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(54) Title: **ANTISENSE IAP NUCLEIC ACIDS AND USES THEREOF**

(57) Abstract: The present invention feature antisense IAP nucleic acids and other negative regulators of the IAP anti-apoptotic pathway, and methods for using them to enhance apoptosis.

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5 ANTISENSE IAP NUCLEIC ACIDS AND USES THEREOF

Field of the Invention

 The invention relates to antisense IAP nucleic acids and methods of using them to increase apoptosis.

10

Background of the Invention

 One way by which cells die is referred to as apoptosis, or programmed cell death. Apoptosis often occurs as a normal part of the development and maintenance of healthy tissues. The process may occur
15 so rapidly that it is difficult to detect.

 The apoptosis pathway is now known to play a critical role in embryonic development, viral pathogenesis, cancer, autoimmune disorders, and neurodegenerative diseases, as well as other events. The failure of an apoptotic response has been implicated in the development of
20 cancer, autoimmune disorders, such as lupus erythematosus and multiple sclerosis, and in viral infections, including those associated with herpes virus, poxvirus, and adenovirus.

 Baculoviruses encode proteins that are termed inhibitors of apoptosis (IAPs) because they inhibit the apoptosis that would otherwise
25 occur when insect cells are infected by the virus. These proteins are thought to work in a manner that is independent of other viral proteins. The baculovirus IAP genes include sequences encoding a ring zinc finger-like motif (RZF), which is presumed to be directly involved in DNA binding, and two N-terminal domains that consist of a 70 amino acid
30 repeat motif termed a BIR domain (Baculovirus IAP Repeat).

The role of apoptosis in cancer has only recently been appreciated. The identification of growth promoting "oncogenes" in the late 1970's gave rise to an almost universal focus on cellular proliferation that dominated research in cancer biology for many years. Long-standing
5 dogma held that anti-cancer therapies preferentially targeted rapidly dividing cancer cells relative to "normal" cells. This explanation was not entirely satisfactory, since some slow growing tumors are easily treated, while many rapidly dividing tumor types are extremely resistant to anti-cancer therapies. Progress in the cancer field has now led to a new
10 paradigm in cancer biology wherein neoplasia is viewed as a failure to execute normal pathways of programmed cell death. Normal cells receive continuous feedback from their neighbors through various growth factors, and commit "suicide" if removed from this context. Cancer cells somehow ignore these commands and continue inappropriate proliferation.
15 Cancer therapies, including radiation and many chemotherapies, have traditionally been viewed as causing overwhelming cellular injury. New evidence suggests that cancer therapies actually work by triggering apoptosis.

Both normal cell types and cancer cell types display a wide range
20 of susceptibility to apoptotic triggers, although the determinants of this resistance are only now under investigation. Many normal cell types undergo temporary growth arrest in response to a sub-lethal dose of radiation or cytotoxic chemical, while cancer cells in the vicinity undergo apoptosis. This provides the crucial treatment "window" of appropriate
25 toxicity that allows successful anti-cancer therapy. It is therefore not surprising that resistance of tumor cells to apoptosis is emerging as a major category of cancer treatment failure.

Compared to the numerous growth-promoting oncogenes identified to date (>100), relatively few genes have been isolated that regulate

apoptosis. The Bcl-2 gene was first identified as an oncogene associated with the development of follicular lymphomas. In contrast to all other oncogenes identified to date, Bcl-2 displays no ability to promote cell proliferation, and instead has been demonstrated to suppress apoptosis by a variety of triggers. Elevated Bcl-2 expression is associated with a poor prognosis in neuroblastoma, prostate and colon cancer, and can result in a multidrug resistant phenotype *in vitro*. Although the study of Bcl-2 has helped revolutionize cancer paradigms, the vast majority of human malignancies do not demonstrate aberrant Bcl-2 expression.

10 In contrast to the findings with Bcl-2, mutation of the p53 tumor suppresser gene has been estimated to occur in up to 50% of human cancers and is the most frequent genetic change associated with cancer to date. The p53 protein plays a crucial role in surveying the genome for DNA damage. The cell type and degree of damage determines whether the cell will undergo growth arrest and repair, or initiate apoptosis.

15 Mutations in p53 interfere with this activity, rendering the cell resistant to apoptosis by a wide range of cellular insults. Some progress has been made in understanding the molecular biology of p53, but many questions remain. p53 is known to function as a transcription factor, with the ability to positively or negatively regulate the expression of a variety of genes involved in cell cycle control, DNA repair, and apoptosis (including the anti-apoptotic Bcl-2 gene described above and the related pro-apoptotic gene Bax).

20 The drug resistant phenotype conferred by p53 alterations has been linked to Bcl-2/Bax regulation, but this correlation does not hold for most cancer types, leaving open the possibility that other critical genes regulated by p53 remain to be identified.

25

Summary of the Invention

We have discovered that inhibitor of apoptosis (IAP) protein overexpression is associated with a wide range of cancer types including ovarian cancer, adenocarcinoma, lymphoma, and pancreatic cancer. In addition, we have found that nuclear localization, fragmentation of the IAPs, and overexpression of the IAPs in the presence of p53 mutations correlate with a cancer diagnosis, a poor prognosis, and resistance to numerous chemotherapeutic cancer drugs. These discoveries provide diagnostic, prognostic, and therapeutic compounds and methods for the detection and treatment of proliferative diseases. One way in which the expression of an IAP in a cell can be decreased is by administering to the cell a negative regulator of the IAP apoptotic pathway, for example, an antisense nucleic acid.

In general, the invention features methods and reagents useful for inducing apoptosis in a cell. The methods and reagents of the invention are useful in treating cancers, and other proliferative diseases.

In a first aspect, the invention features an inhibitor of apoptosis (IAP) antisense nucleic acid that inhibits IAP biological activity, regardless of the length of the antisense nucleic acid. In preferred embodiments, the IAP is XIAP, HIAP1, or HIAP2. In other preferred embodiments, the antisense nucleic acid is mammalian, for example, mouse or human. In yet another embodiment, the antisense nucleic acid is between 8 and 30 nucleotides in length.

In still other further preferred embodiments, the XIAP antisense is chosen from any one of SEQ ID NOS: 1 through 96, and the HIAP1 antisense is chosen from any one of SEQ ID NOS: 97 through 194. Preferably the IAP biological activity is inhibition of apoptosis or inhibition of IAP RNA or polypeptide expression. The antisense nucleic acid may comprise at least one modified internucleoside linkage.

Preferably the modified internucleoside linkage is a phosphorothioate, a methylphosphonate, a phosphotriester, a phosphorodithioate, or a phosphoselenate linkage. In addition, the antisense nucleic acid may comprise at least one modified sugar moiety. Preferably this modified
5 sugar moiety is a 2'-O methoxyethyl group or a 2'-O methyl group. In still another preferred embodiment, the antisense nucleic acid is a chimeric nucleic acid. Preferably the chimeric nucleic acid comprises DNA residues linked together by phosphorothioate linkages, and the DNA residues are flanked on each side by at least one 2'-O methyl RNA residue
10 linked together by a phosphorothioate linkage. More preferably the DNA residues are flanked on each side by at least three 2'-O methyl RNA residues. In yet another embodiment, the antisense nucleic acid is a ribozyme.

In a second aspect, the invention features a method of enhancing
15 apoptosis in a cell, involving administering to the cell a negative regulator of the IAP-dependent antiapoptotic pathway. In preferred embodiments the negative regulator is an antisense IAP nucleic acid, an antibody that specifically binds an IAP polypeptide, an IAP polypeptide comprising a ring zinc finger, said polypeptide having no more than two BIR domains, a
20 nucleic acid encoding the ring zinc finger domain of an IAP polypeptide, or a compound that prevents cleavage of the IAP polypeptide.

In preferred embodiments of the second aspect of the invention, the cell is in a mammal diagnosed with a proliferative disease, for example, cancer. The cell may comprises a mucosa-associated lymphoid tissue
25 (MALT), a tissue in which the IAP gene HIAP1 is frequently involved in a translocation, resulting in marginal zone cell lymphomas. The cell may also be a breast cancer cell, where increased HIAP1 expression is known to correlate with tumor progression. The cell may also be a cell in which NFkB expression or activity is increased, for example, cell of head and

neck carcinomas, adult T-cell lymphomas, nasopharyngeal carcinomas, and Hodgkin's disease. The cell may also be an acute myelogenous leukemia cell, where increased XIAP levels correlate with poor patient prognosis. In addition, the cell may be a small cell lung carcinoma cell,
5 where increased levels of XIAP correlates with increased resistance to radiation treatment.

In preferred embodiments of the second aspect of the invention, the IAP is XIAP, HIAP1, or HIAP2. Preferably the antisense nucleic acid is mammalian, for example, mouse or human. In still other preferred
10 embodiments, the XIAP antisense is chosen from any one of SEQ ID NOS: 1 through 96, and the HIAP1 antisense is chosen from any one of SEQ ID NOS: 97 through 194.

In still other embodiments of the second aspect of the invention, the antisense nucleic acid comprises at least one modified internucleoside
15 linkage. Preferably the modified internucleoside linkage is a phosphorothioate, a methylphosphonate, a phosphotriester, a phosphorodithioate, or a phosphoselenate linkage. In addition, the antisense nucleic acid may comprise at least one modified sugar moiety. Preferably this modified sugar moiety is a 2'-O methoxyethyl group or a
20 2'-O methyl group. In still another preferred embodiment, the antisense nucleic acid is a chimeric nucleic acid. Preferably the chimeric nucleic acid comprises DNA residues linked together by phosphorothioate linkages, and the DNA residues are flanked on each side by at least one 2'-O methyl RNA residue linked together by a phosphorothioate linkage.
25 More preferably the DNA residues are flanked on each side by at least three 2'-O methyl RNA residues. In still further embodiments, administration of the antisense nucleic acid sensitizes the cell to chemotherapy or radiotherapy. In addition, the cell may be *in vitro* or *in vivo*.

In a third aspect, the invention features a pharmaceutical composition comprising a mammalian IAP antisense nucleic acid. In one preferred embodiment, the mammalian antisense IAP nucleic acid is a human antisense nucleic acid. Preferably the antisense nucleic acid binds
5 a target sequence of the human XIAP gene or mRNA, the human HIAP1 gene or mRNA, the human HIAP2 gene or mRNA, the murine XIAP gene or mRNA, the murine HIAP1 gene or mRNA, or the murine HIAP2 gene or mRNA. More preferably the composition comprises an antisense nucleic acid chosen from any one of SEQ ID NOS: 1 through 96 (XIAP)
10 or SEQ ID NOS: 97 through 194 (HIAP1).

In another aspect, the invention features an IAP gene nucleic acid fragment or antisense RNA sequence for use in suppressing cell proliferation. Such nucleic acids of the invention and methods for using them may be identified according to a method involving: (a) providing a
15 cell sample; (b) introducing by transformation into the cell sample a candidate IAP nucleic acid; (c) expressing the candidate IAP nucleic acid within the cell sample; and (d) determining whether the cell sample exhibits an altered apoptotic response, whereby decreased apoptosis identifies an anti-proliferative compound. Preferably the cell is a cancer
20 cell.

In another aspect, the invention features a method of treating a patient diagnosed with a proliferative disease. In the method, apoptosis may be induced in a cell to control a proliferative disease either alone or in combination with other therapies by administering to the cell a negative
25 regulator of the IAP-dependent or anti-apoptotic pathway. The negative regulator may be, but is not limited to, an IAP ring zinc finger, and an IAP polypeptide that includes a ring zinc finger and lacks at least one BIR domain. Alternatively, apoptosis may be induced in the cell by administering a nucleic acid encoding an IAP antisense RNA molecule

administered directly or via gene therapy (see U.S. Pat. No. 5,576,208 for general parameters that may be applicable in the selection of IAP antisense RNAs). In yet another method, the negative regulator may be a purified antibody, or a fragment thereof, that binds specifically to an IAP polypeptide. For example, in one preferred embodiment, the antibody may bind to an approximately 26 kDa cleavage product of an IAP polypeptide that includes at least one BIR domain but lacks a ring zinc finger domain.

In two additional aspects, the invention features a transgenic animal and methods of using the mammal for detection of anti-cancer therapeutics. Preferably the mammal overexpresses an IAP polypeptide and/or expresses an IAP antisense RNA or IAP fragment. In one embodiment, the animal also has a genetic predisposition to cancer or has cancer cells under conditions that provide for proliferation absent the transgenic construct encoding either the antisense RNA or fragment.

“Protein” or “polypeptide” or “polypeptide fragment” means any chain of more than two amino acids, regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally-occurring polypeptide or peptide, or constituting a non-naturally occurring polypeptide or peptide.

“Apoptosis” means the process of cell death wherein a dying cell displays a set of well-characterized biochemical hallmarks that include cell membrane blebbing, cell soma shrinkage, chromatin condensation, and DNA laddering. Cells that die by apoptosis include neurons (e.g., during the course of neurodegenerative diseases such as stroke, Parkinson’s disease, and Alzheimer’s disease), cardiomyocytes (e.g., after myocardial infarction or over the course of congestive heart failure), and cancer cells (e.g., after exposure to radiation or chemotherapeutic agents). Environmental stress (e.g., hypoxic stress) that is not alleviated may cause

a cell to enter the early phase of the apoptotic pathway, which is reversible (i.e., cells at the early stage of the apoptotic pathway can be rescued). At a later phase of apoptosis (the commitment phase), cells cannot be rescued, and, as a result, are committed to die.

5 Proteins and compounds known to stimulate and inhibit apoptosis in a diverse variety of cells are well known in the art. For example, intracellular expression and activation of the caspase (ICE) family induces or stimulates apoptotic cell death, whereas expression of the IAPs or some members of the Bcl-2 family inhibits apoptotic cell death. In addition,
10 there are survival factors that inhibit cell death in specific cell types. For example, neurotrophic factors, such as NGF inhibit neuronal apoptosis.

 In some situations it may be desirable to artificially stimulate or inhibit apoptotic cell death by gene therapy or by a compound that mimics a gene therapeutic effect. For example, a cell that is susceptible to
15 apoptosis induced by disease or environmental stress may be made more resistant to apoptosis by introducing an expression vector encoding an anti-apoptotic protein (such as an IAP, a Bcl-2 family member, or a neurotrophin) into the cell. Conversely, a cancer cell may be made less resistant to apoptosis by introducing into it an expression vector encoding
20 a pro-apoptotic protein (such as a caspase) or by introducing into it an antisense nucleic acid, for example, an IAP antisense nucleic acid, regardless of its length. In addition, placement of the encoded protein of interest under the translational regulation of a XIAP IRES ensures that copious quantities of the protein are produced, especially under cellular
25 conditions during which most protein translation (i.e., cap-dependent protein translation) is down-regulated, e.g., when a cell is under environmental stress, and when a cell is at a threshold for entering the apoptotic pathway.

By "IAP gene" is meant a gene encoding a polypeptide having at least one BIR domain and a ring zinc finger domain that is capable of modulating (inhibiting or enhancing) apoptosis in a cell or tissue when provided by other intracellular or extracellular delivery methods (see, e.g.,
 5 U.S. Patent No. 5,919,912, U.S.S.N. 08/576,965, and PCT/IB96/01022). In preferred embodiments, the IAP gene is a gene having about 50% or greater nucleotide sequence identity to at least one of the IAP amino acid encoding sequences of Figs. 1 through 6, or portions thereof. Preferably the region of sequence over which identity is measured is a region
 10 encoding at least one BIR domain and a ring zinc finger domain. Mammalian IAP genes include nucleotide sequences isolated from any mammalian source. Preferably the mammal is a human.

The term "IAP gene" is meant to encompass any member of the family of genes that encode inhibitors of apoptosis. An IAP gene may
 15 encode a polypeptide that has at least 20%, preferably at least 30%, and most preferably at least 50% amino acid sequence identity with at least one of the conserved regions of one of the IAP members described herein (i.e., either the BIR or ring zinc finger domains from human or murine XIAP, HIAP1, and HIAP2). Representative members of the IAP gene
 20 family include, without limitation, the human and murine XIAP, HIAP1, and HIAP2 genes.

By "IAP protein" or "IAP polypeptide" is meant a polypeptide, or fragment thereof, encoded by an IAP gene.

By "BIR domain" is meant a domain having the amino acid
 25 sequence of the consensus sequence: Xaa1-Xaa1-Xaa1-Arg-Leu-Xaa1-Thr-Phe-Xaa1-Xaa1-Trp-Pro-Xaa2-Xaa1-Xaa1-Xaa2-Xaa2-Xaa1-Xaa1-Xaa1-Xaa1-Leu-Ala-Xaa1-Ala-Gly-Phe-Tyr-Tyr-Xaa1-Gly-Xaa1-Xaa1-Asp-Xaa1-Val-Xaa1-Cys-Phe-Xaa1-Cys-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Trp-Xaa1-Xaa1-Xaa1-Asp-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-Xaa1-His-Xaa1-Xaa1-Xaa1-Xaa1-

Pro-Xaal-Cys-Xaal-Phe-Val, wherein Xaal is any amino acid and Xaa2 is any amino acid or is absent (SEQ ID NO: 216). Preferably the sequence is substantially identical to one of the BIR domain sequences provided for XIAP, HIAP1, or HIAP2 herein.

5 By "ring zinc finger" or "RZF" is meant a domain having the amino acid sequence of the consensus sequence: Glu-Xaal-Xaal-Xaal-Xaal-Xaal-Xaal-Xaa2-Xaal-Xaal-Xaal-Cys- Lys-Xaa3-Cys-Met-Xaal-Xaal-Xaal-Xaal-Xaa3-Xaal-Phe-Xaal-Pro-Cys-Gly-His-Xaal-Xaal-Xaal-Cys-Xaal-Xaal-Cys-Ala- Xaal-Xaal-Xaal-Xaal-Xaal-Cys-Pro-Xaal-
10 Cys, wherein Xaal is any amino acid, Xaa2 is Glu or Asp, and Xaa3 is Val or Ile (SEQ ID NO: 217).

Preferably the sequence is substantially identical to the RZF domains provided in U.S.S.N. 08/800,929, incorporated herein by reference, for the human or murine XIAP, HIAP1, or HIAP2.

15 By "enhancing apoptosis" is meant increasing the number of cells that apoptose in a given cell population. Preferably the cell population is selected from a group including ovarian cancer cells, breast cancer cells, pancreatic cancer cells, T cells, neuronal cells, fibroblasts, or any other cell line known to proliferate in a laboratory setting. It will be appreciated
20 that the degree of apoptosis enhancement provided by an apoptosis-enhancing compound in a given assay will vary, but that one skilled in the art can determine the statistically significant change in the level of apoptosis that identifies a compound that enhances apoptosis otherwise limited by an IAP. Preferably "enhancing apoptosis" means that the
25 increase in the number of cells undergoing apoptosis is at least 25%, more preferably the increase is 50%, and most preferably the increase is at least one-fold. Preferably the sample monitored is a sample of cells that normally undergo insufficient apoptosis (i.e., cancer cells). Methods for

detecting a changes in the level of apoptosis (i.e., enhancement or reduction) are described herein.

By "proliferative disease" is meant a disease that is caused by or results in inappropriately high levels of cell division, inappropriately low
5 levels of apoptosis, or both. For example, cancers such as lymphoma, leukemia, melanoma, ovarian cancer, breast cancer, pancreatic cancer, and lung cancer are all examples of proliferative disease.

By "IAP biological activity" is meant any activity known to be caused *in vivo* or *in vitro* by an IAP polypeptide.

10 By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding (as used herein) an IAP polypeptide.

By "transgene" is meant any piece of DNA that is inserted by
15 artifice into a cell, and becomes part of the genome of the organism that develops from that cell. Such a transgene may include a gene that is partly or entirely heterologous (i.e., foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism.

By "transgenic" is meant any cell that includes a DNA sequence
20 that is inserted by artifice into a cell and becomes part of the genome of the organism that develops from that cell. As used herein, the transgenic organisms are generally transgenic mammals (e.g., rodents, such as rats or mice) and the DNA (transgene) is inserted by artifice into the nuclear genome.

25 By "transformation" is meant any method for introducing foreign molecules, for example, an antisense nucleic acid, into a cell. Lipofection, calcium phosphate precipitation, retroviral delivery, electroporation, biolistic transformation, and penetratin are just a few of the teachings that may be used. For example, biolistic transformation is a method for

introducing foreign molecules into a cell using velocity driven microprojectiles such as tungsten or gold particles. Such velocity-driven methods originate from pressure bursts that include, but are not limited to, helium-driven, air-driven, and gunpowder-driven techniques. Biolistic transformation may be applied to the transformation or transfection of a wide variety of cell types and intact tissues including, without limitation, intracellular organelles (e.g., and mitochondria and chloroplasts), bacteria, yeast, fungi, algae, animal tissue, and cultured cells. In another example, a foreign molecule (e.g., an antisense nucleic acid) can be translocated into a cell using the penetratin system as described, for example, by Prochiantz (Nature Biotechnology 16: 819-820, 1998; and Derossi et al. (Trends Cell Biol. 8: 84-87, 1998). In this system a penetratin peptide contains a transduction sequence that carries the peptide and a conjugated partner, for example, a phosphorothioate antisense nucleic acid (that is cross-linked through a disulfide bridge to the peptide) across the plasma membrane into the cell. The disulfide band is reduced inside the cell, releasing the partner.

By "antisense," as used herein in reference to nucleic acids, is meant a nucleic acid sequence, regardless of length, that is complementary to the coding strand or mRNA of an IAP gene. Preferably the antisense nucleic acid is capable of enhancing apoptosis when present in a cell that normally does not undergo sufficient apoptosis. Preferably the increase is at least 10%, relative to a control, more preferably 25%, and most preferably 1-fold or more. Preferably an IAP antisense nucleic acid comprises from about 8 to 30 nucleotides. An IAP antisense nucleic acid may also contain at least 40, 60, 85, 120, or more consecutive nucleotides that are complementary to a IAP mRNA or DNA, and may be as long as a full-length IAP gene or mRNA. The antisense nucleic acid may contain a modified backbone, for example, phosphorothioate, phosphorodithioate, or

other modified backbones known in the art, or may contain non-natural internucleoside linkages.

By "ribozyme" is meant an RNA that has enzymatic activity, possessing site specificity and cleavage capability for a target RNA molecule. Ribozymes can be used to decrease expression of a polypeptide. Methods for using ribozymes to decrease polypeptide expression are described, for example, by Turner et al., (Adv. Exp. Med. Biol. 465:303-318, 2000) and Norris et al., (Adv. Exp. Med. Biol. 465:293-301, 2000).

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% homology to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

Sequence identity is typically measured using sequence analysis software with the default parameters specified therein (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). This software program matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "substantially pure polypeptide" is meant a polypeptide that has been separated from the components that naturally accompany it.

Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably the polypeptide is an IAP polypeptide that is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, pure. A substantially pure IAP polypeptide may be obtained, for example, by extraction from a natural source (e.g., a fibroblast, neuronal cell, or lymphocyte) by expression of a recombinant nucleic acid encoding an IAP polypeptide, or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A protein is substantially free of naturally associated components when it is separated from those contaminants that accompany it in its natural state. Thus, a protein that is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes.

By "substantially pure DNA" is meant DNA that is free of the genes that, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other

sequences. It also includes a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence, that directs transcription and translation of the sequence (i.e., facilitates the production of, e.g., an IAP polypeptide, a recombinant protein or an RNA molecule).

By "reporter gene" is meant a gene whose expression may be assayed; such genes include, without limitation, glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), and Beta-galactosidase.

By "promoter" is meant a minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements that are sufficient to render promoter-dependent gene expression controllable for cell type-specific, tissue-specific or that are inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the native gene.

By "operably linked" is meant that a gene and one or more regulatory sequences are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequences.

By "conserved region" is meant any stretch of six or more contiguous amino acids exhibiting at least 30%, preferably 50%, and most preferably 70% amino acid sequence identity between two or more of the IAP family members, (e.g., between human HIAP1, HIAP2, and XIAP). Examples of preferred conserved regions include, without limitation, BIR domains and ring zinc finger domains.

By "detectably-labelled" is meant any means for marking and identifying the presence of a molecule, e.g., an oligonucleotide probe or primer, a gene or fragment thereof, or a cDNA molecule. Methods for detectably-labelling a molecule are well known in the art and include,

without limitation, radioactive labelling (e.g., with an isotope such as ^{32}P or ^{35}S) and nonradioactive labelling (e.g., chemiluminescent labeling or fluorescein labelling).

By "purified antibody" is meant an antibody that is at least 60%, by weight, free from proteins and naturally occurring organic molecules with which it is naturally associated. Preferably the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, e.g., an IAP-specific antibody. A purified antibody may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques.

By "specifically binds" is meant an antibody that recognizes and binds a protein but that does not substantially recognize and bind other molecules in a sample, e.g., a biological sample, that naturally includes protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

Fig. 1 is the human XIAP cDNA sequence (SEQ ID NO: 218) and the XIAP polypeptide sequence (SEQ ID NO: 219).

Fig. 2 is the human HIAP1 cDNA sequence (SEQ ID NO: 220) and the HIAP1 polypeptide sequence (SEQ ID NO: 221).

Fig. 3 is the human HIAP2 cDNA sequence (SEQ ID NO: 222) and the HIAP2 polypeptide sequence (SEQ ID NO: 223). The sequence absent in the HIAP2 variant is boxed.

Fig. 4 is the murine XIAP (also referred to as "miap-3") cDNA sequence (SEQ ID NO: 224) and encoded murine XIAP polypeptide sequence (SEQ ID NO: 225).

Fig. 5 is the murine HIAP1 (also referred to as "miap-1") cDNA sequence (SEQ ID NO: 226) and the encoded murine HIAP1 polypeptide sequence (SEQ ID NO: 227).

Fig. 6 is the murine HIAP2 (also referred to as "miap-2") cDNA
5 sequence (SEQ ID NO: 228) and the encoded murine HIAP2 polypeptide (SEQ ID NO: 229).

Figs. 7A through 7L are graphs showing the effect of antisense XIAP oligonucleotides on XIAP protein expression, relative to total protein (Figs. 7A, 7C, 7E, 7G, 7I, and 7K). Figs. 7B, 7D, 7F, 7H, 7J, and
10 7L are the total protein concentration values for each oligonucleotide transfection compared to mock transfection results that were used to normalize the above XIAP protein results.

Figs. 8A through 8C are graphs showing the effects of various antisense XIAP oligonucleotides, alone or in combination, on XIAP RNA (Fig. 8A) and protein (Fig. 8B). Fig. 8C is a graph of the total protein
15 concentration values for each oligonucleotide transfection compared to mock transfection results, which were used to normalize the XIAP protein results shown in Fig. 8B.

Figs. 9A through 9D are graphs of the effects of antisense XIAP
20 oligonucleotides on cell viability (Figs. 9A, 9C, and 9D), and chemosensitization in the presence of adriamycin (Fig. 9B).

Fig. 10 is a graph showing the effects of HIAP1 antisense oligonucleotides on HIAP1 RNA levels.

Fig. 11A is a densitometric scan of a Western blot showing the
25 effects of HIAP1 antisense oligonucleotides on a cell's ability to block cycloheximide-induced upregulation of HIAP1 protein.

Fig. 11 is a graph showing the effects of HIAP1 antisense oligonucleotides on a cell's ability to block cycloheximide-induced upregulation of HIAP1 protein.

Fig. 12 is a graph showing the effects of HIAP1 antisense oligonucleotides on cytotoxicity, as measured by total protein.

Fig. 13 is a graph showing the validation of the sequence specificity for HIAP1 antisense oligonucleotide APO 2.

5 Fig. 14 is a graph showing the effect of HIAP1 antisense oligonucleotides on the chemosensitization of drug-resistant SF295 glioblastomas.

Fig. 15 is the human XIAP sequence containing a 5' UTR, the coding region, and a 3' UTR (SEQ ID NO: 230).

10 Fig. 16 is the human HIAP1 sequence containing a 5' UTR, the coding region, and a 3' UTR (SEQ ID NO: 231).

Detailed Description of the Invention

The present invention provides IAP antisense nucleic acid
15 sequences that inhibit IAP biological activity, regardless of length, and methods for using them to induce apoptosis in a cell. The antisense nucleic acids of the present invention may also be used to form pharmaceutical compositions. The invention also features methods for enhancing apoptosis in a cell by administering a negative regulator of the
20 IAP anti-apoptotic pathway other than antisense. Such negative regulators include, for example, an IAP polypeptide comprising a ring zinc finger having no more than two BIR domains, and a compound that prevents cleavage of an IAP polypeptide. Such negative regulators may also be used to form a pharmaceutical composition. These pharmaceutical
25 compositions may be used to treat, ameliorate, improve, sustain, or prevent a proliferative disease, for example, cancer, or a symptom of a proliferative disease.

Administration

An IAP antisense nucleic acid, or other negative regulator of the IAP anti-apoptotic pathway may be administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the compounds to patients suffering from a disease that is caused by excessive cell proliferation. Administration may begin before the patient is symptomatic. Any appropriate route of administration may be employed, for example, administration may be parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, suppository, or oral administration. For example, therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found, for example, in "Remington's Pharmaceutical Sciences." Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for IAP modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example,

polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

The formulations can be administered to human patients in therapeutically effective amounts (e.g., amounts which prevent, eliminate,
5 or reduce a pathological condition) to provide therapy for a disease or condition. The preferred dosage of therapeutic agent to be administered is likely to depend on such variables as the type and extent of the disorder, the overall health status of the particular patient, the formulation of the compound excipients, and its route of administration.

10 If desired, treatment with an IAP antisense nucleic acid, IAP fragments, or other negative regulator of the anti-apoptotic pathway may be combined with more traditional therapies for the proliferative disease such as surgery or chemotherapy.

For any of the methods of application described above, the
15 therapeutic antisense IAP nucleic acid or other negative regulator of the IAP anti-apoptotic pathway is preferably applied to the site of the needed apoptosis event (for example, by injection). However, it may also be applied to tissue in the vicinity of the predicted apoptosis event or to a blood vessel supplying the cells predicted to require enhanced apoptosis.

20 The dosage of an antisense IAP nucleic acid, or a negative regulator of the IAP anti-apoptotic pathway, for example, an IAP fragment, IAP mutant protein or an IAP antibody depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1 mg and 100 mg inclusive are administered per day to an adult
25 in any pharmaceutically acceptable formulation. In addition, treatment by any IAP-modulating gene therapy approach may be combined with more traditional therapies.

Antisense Therapy

Anti-cancer therapy may be accomplished by direct administration of a therapeutic antisense IAP nucleic acid to a cell that is expected to require enhanced apoptosis. The antisense nucleic acid may be produced
5 and isolated by any one of many standard techniques. Administration of IAP antisense nucleic acids to malignant cells can be carried out by any of the methods for direct nucleic acid administration, as described herein.

Retroviral vectors, adenoviral vectors, adeno-associated viral vectors, or other viral vectors with the appropriate tropism for cells likely
10 requiring enhanced apoptosis (for example, breast cancer and ovarian cancer cells) may be used as a gene transfer delivery system for a therapeutic antisense IAP gene construct. Numerous vectors useful for this purpose are generally known (Miller, Human Gene Therapy 15-14, 1990; Friedman, Science 244:1275-1281, 1989; Eglitis and Anderson,
15 BioTechniques 6:608-614, 1988; Tolstoshev and Anderson, Current Opinion in Biotechnology 1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416, 1991; Miller et al., BioTechniques 7:980-990, 1989; Le
20 Gal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S-83S, 1995).

Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Patent No. 5,399,346). Non-viral approaches may
25 also be employed for the introduction of therapeutic DNA into cells otherwise predicted to undergo apoptosis. For example, IAPs may be introduced into a cell by lipofection (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413, 1987; Ono et al., Neurosci. Lett. 117:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al., Meth. Enz. 101:512,

1983), the penetratin system (Allinquant et al., J. Cell Biol. 128:919-927, 1995; Prochiantz, Curr. Opin. Neurobiol. 6:629-634, 1996), asialorosonucoid-polylysine conjugation (Wu et al., J. Biol. Chem. 263:14621, 1988; Wu et al., J. Biol. Chem. 264:16985, 1989); or, less
5 preferably microinjection under surgical conditions (Wolff et al., Science 247:1465, 1990).

In the therapeutic nucleic acid constructs described, nucleic acid expression can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein
10 promoters), and regulated by any appropriate mammalian regulatory element. For example, if desired, enhancers known to preferentially direct gene expression in ovarian cells, breast tissue, neural cells, T cells, or B cells may be used to direct expression. Enhancers include, without
15 limitation, those that are characterized as tissue- or cell-specific in their expression. Alternatively, if a clone is used as a therapeutic construct, regulation may be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

20 Therapeutic Products

For IAP related therapies one may employ the paradigms utilized for Bcl-2 and Ras antisense development, although accommodation of an IAP mutation is not required (in contrast to Ras antisense). Most useful are antisense constructs that enhance apoptosis at least 10%, preferably by
25 enhancing degradation of the RNA in the nucleus.

Manipulation of cancer chemotherapeutic drug resistance using an antisense oligonucleotide and fragment approaches

We have documented that overexpression of the IAPs renders cell lines resistant to serum growth factor withdrawal, tumor necrosis factor alpha (TNF) and menadione exposure, all of which are treatments that normally induce apoptosis. Herein, we describe the extension of these studies to cancer cell lines using apoptotic triggers used in clinical situations, such as doxorubicin, adriamycin, and methotrexate. Our findings have led up to the design of antisense RNA therapeutics. Rapid screening of multiple cell lines for apoptotic response has been made feasible through the generation of a series of sense and antisense adenoviral IAP and expression vectors, as well as control lacZ viruses. One may now show enhanced drug resistance using the expression constructs. In addition, anti-sense adenovirus constructs may be developed and used to test reversal of the drug resistant phenotype of appropriate cell lines. We have designed a series of antisense oligonucleotides to various regions of each of the *iaps*. These oligonucleotides may be used to enhance drug sensitivity after testing in an assay system, i.e., with the adenoviral vectors system. Animal modeling of the effectiveness of antisense IAP oligonucleotides may also be employed as a step in testing and appropriate transgenic mammals for this are described in U.S.S.N. 08/800,929, incorporated herein by reference, and are also generally available in the art.

Characterization of IAP Activity and Intracellular Localization Studies

The ability of IAPs to modulate apoptosis can be defined *in vitro* systems in which alterations of apoptosis can be detected. Mammalian expression constructs carrying IAP cDNAs, which are either full-length, truncated, or antisense constructs can be introduced into cell lines, such as

CHO, NIH 3T3, HL60, Rat-1, or Jurkat cells. In addition, SF21 insect cells may be used, in which case the IAP gene is preferentially expressed using an insect heat shock promoter. Following transfection, apoptosis can be induced by standard methods, which include serum withdrawal, or
5 application of staurosporine, menadione (which induces apoptosis via free radical formation), or anti-Fas antibodies. As a control, cells are cultured under the same conditions as those induced to undergo apoptosis, but either not transfected, or transfected with a vector that lacks an IAP insert. The ability of each IAP related construct to inhibit or enhance apoptosis
10 upon expression can be quantified by calculating the survival index of the cells, i.e., the ratio of surviving transfected cells to surviving control cells. These experiments can confirm the presence of apoptosis inhibiting activity and, as discussed below, can also be used to determine the functional region(s) of an IAP that may be employed to achieve
15 enhancement of apoptosis. These assays may also be performed in combination with the application of additional compounds in order to identify compounds that enhance apoptosis via IAP expression.

Apoptosis Assays

20 Specific examples of apoptosis assays are provided in the following references. Assays for apoptosis in lymphocytes are disclosed by: Li et al., "Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein", Science 268:429-431, 1995; Gibellini et al., "Tat-expressing Jurkat cells show an increased resistance to different apoptotic stimuli,
25 including acute human immunodeficiency virus-type 1 (HIV-1) infection", Br. J. Haematol. 89:24-33, 1995; Martin et al., "HIV-1 infection of human CD4⁺ T cells *in vitro*. Differential induction of apoptosis in these cells." J. Immunol. 152:330-342, 1994; Terai et al., "Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1", J.

Clin. Invest. 87:1710-1715, 1991; Dhein et al., "Autocrine T-cell suicide mediated by APO-1/(Fas/CD95)", *Nature* 373:438-441, 1995; Katsikis et al., "Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals", *J. Exp. Med.* 1815:2029-2036, 1995; Westendorp et al., "Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120", *Nature* 375:497, 1995; and DeRossi et al., *Virology* 198:234-44, 1994.

Assays for apoptosis in fibroblasts are disclosed by: Vossbeck et al., "Direct transforming activity of TGF-beta on rat fibroblasts", *Int. J. Cancer* 61:92-97, 1995; Goruppi et al., "Dissection of c-myc domains involved in S phase induction of NIH3T3 fibroblasts", *Oncogene* 9:1537-1544, 1994; Fernandez et al., "Differential sensitivity of normal and Ha-ras transformed C3H mouse embryo fibroblasts to tumor necrosis factor: induction of bcl-2, c-myc, and manganese superoxide dismutase in resistant cells", *Oncogene* 9:2009-2017, 1994; Harrington et al., "c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines", *EMBO J.*, 13:3286-3295, 1994; and Itoh et al., "A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen", *J. Biol. Chem.* 268:10932-10937, 1993.

Assays for apoptosis in neuronal cells are disclosed by: Melino et al., "Tissue transglutaminase and apoptosis: sense and antisense transfection studies with human neuroblastoma cells", *Mol. Cell. Biol.* 14:6584-6596, 1994; Rosenbaum et al., "Evidence for hypoxia-induced, programmed cell death of cultured neurons", *Ann. Neurol.* 36:864-870, 1994; Sato et al., "Neuronal differentiation of PC12 cells as a result of prevention of cell death by bcl-2", *J. Neurobiol.* 25:1227-1234, 1994; Ferrari et al., "N-acetylcysteine D- and L-stereoisomers prevents apoptotic death of neuronal cells", *J. Neurosci.* 15:2857-2866, 1995; Talley et al., "Tumor necrosis factor alpha-induced apoptosis in human neuronal cells:

protection by the antioxidant N-acetylcysteine and the genes bcl-2 and crmA", Mol. Cell Biol. 15:2359-2366, 1995; and Walkinshaw et al., "Induction of apoptosis in catecholaminergic PC12 cells by L-DOPA. Implications for the treatment of Parkinson's disease.", J. Clin. Invest. 5 95:2458-2464, 1995.

Assays for apoptosis in insect cells are disclosed by: Clem et al., "Prevention of apoptosis by a baculovirus gene during infection of insect cells", Science 254:1388-1390, 1991; Crook et al., "An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif", J. Virol. 10 67:2168-2174, 1993; Rabizadeh et al., "Expression of the baculovirus p35 gene inhibits mammalian neural cell death", J. Neurochem. 61:2318-2321, 1993; Birnbaum et al., "An apoptosis inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs", J. Virol. 68:2521-2528, 1994; and Clem et al., "Control of programmed 15 cell death by the baculovirus genes p35 and IAP", Mol. Cell. Biol. 14:5212-5222, 1994.

The following examples are to illustrate the invention. They are not meant to limit the invention in any way.

20

Example 1: Testing of antisense oligonucleotides

1. *Complete panel of adenovirus constructs.* The panel may consist of approximately four types of recombinant virus. A) Sense orientation 25 viruses for each of the IAP open reading frames. These viruses are designed to massively overexpress the recombinant protein in infected cells. XIAP, HIAP1, HIAP2, and NAIP. B) Antisense orientation viruses in which the viral promoter drives the synthesis of an mRNA of opposite polarity to the *iap* mRNA, thereby shutting off host cell synthesis of the

targeted protein coding region. XIAP, HIAP1, HIAP2, and NAIP
“antisense” constructs are used for production of such antisense IAPs. C)
Sub-domain expression viruses. These constructs express only a partial
IAP protein in infected cells. We have data indicating that deletion of the
5 zinc finger of XIAP renders the protein more potent in protecting cell
against apoptotic triggers. This data also indicates that expression of the
zinc finger alone will indicate apoptosis by functioning as a dominant-
negative repressor of XIAP function. XIAP- ZF and XIAP- BIR viruses
are required. D) Control viruses. Functional analysis of the IAPs requires
10 suitable positive and negative controls for comparison. Bcl-2 sense, Bcl-2
antisense, p53 sense, and Lac Z (negative control) viruses may be utilized.

2. *Confirmation of recombinant adenovirus function.* Verification of the
sense adenovirus function involves infection of tissue culture cells and
15 determination of protein expression levels. We have performed Western
blot analysis of several of the recombinant adenoviruses, including NAIP,
XIAP and XIAP- ZF. The remaining viruses may be readily assessed for
protein expression using the polyclonal IAP antibodies. Functional
analysis of the antisense viruses may be done at the RNA level using
20 either Northern blots of total RNA harvested from infected tissue culture
cells or ribonuclease protection assays. Western blot analysis of infected
cells will be used to determine whether the expressed antisense RNA
interferes with IAP expression in the host cell.

25 3. *Documentation that IAP overexpression results in increased drug
resistance.* We have optimized cell death assays to allow high through-put
of samples with minimal sample variation. Testing of the sense IAP
adenoviruses for their ability to alter drug sensitivity of breast and
pancreatic adenocarcinoma cell lines may be accomplished as follows.

Cancer cell lines are infected with the recombinant viruses, cultured for 5 days, then subdivided into 24 well plates. Triplicate cell samples each receive increasing concentrations of the anti-cancer drug under investigation. Samples are harvested at 24, 48, and 72 hours post-
5 exposure, and assayed for the number of viable cells in the well. The dose response curve is then compared to uninfected and control virus (both positive and negative) infected cells. One may document a dramatic increase in the relative resistance of the cancer cell lines when infected with the sense viruses, confirming our hypothesis that overexpression of
10 the IAP proteins contributes to the anti-apoptotic phenotype of cancer cells. Initial experiments utilize the drugs doxorubicin and adriamycin.

4. Documentation that antisense IAP overexpression results in increased drug sensitivity. Having confirmed that IAP overexpression renders
15 cancer cells more resistant to chemotherapeutic drugs, one may examine whether the antisense adenoviruses render the same cells more sensitive. The effectiveness of antisense IAP viruses relative to antisense Bcl-2 virus will also be assessed as a crucial milestone.

5. Identification of antisense oligonucleotides. Concomitant to the
20 adenovirus work, we have designed a series of antisense oligonucleotides to various regions of each of the IAPs. A generally accepted model of how antisense oligonucleotides function proposes that the formation of RNA/DNA duplexes in the nucleus activates cellular RnaseH enzymes
25 which then enzymatically degrade the mRNA component of the hybrid. Virtually any region of the mRNA can be targeted, and therefore choosing an appropriate sequence to target is somewhat empirical.

6. *Optimization of oligonucleotides.* A secondary round of oligonucleotides may be made when effective target regions have been identified. These oligonucleotides target sequences in the immediate vicinity of the most active antisense oligonucleotides identified using methods such as those provided above. A second round of testing by Northern blot analysis may be required.

7. *Testing antisense oligonucleotides in vitro.* Following successful identification and optimization of targeting oligonucleotides, one may test these in the tissue culture model system using the optimal cell lines such as those described in the cancer survey described in U.S.S.N. 08/800,929, incorporated herein by reference. Experimental procedures may parallel those used in the recombinant antisense adenovirus work. Negative control oligonucleotides with miss-match sequences are used to establish base line or non-specific effects. Assisted transfection of the oligonucleotides using cationic lipid carriers may be compared to unassisted transfection. Confirmation of the effectiveness of specific antisense oligonucleotides prompts synthesis of oligonucleotides with modified phosphodiester linkages, such as phosphorothioate or methylimino substituted oligonucleotides. These may also be tested *in vitro*.

8. *Animal modeling of antisense oligonucleotide therapies.* Animal modeling of the effectiveness of the antisense IAP approach is described here. Cell lines are routinely assessed for their tumorigenic potential in "nude" mice, a hairless strain of mouse that is immunocompromised, and thus extremely susceptible to developing tumors. In the nude mouse assay, cancer cells are grown in tissue culture and then injected under the skin at multiple sites. The frequency with which these cells give rise to

palpable tumors within a defined period of time provides an index of the tumorigenic potential of the cell line in the absence of interference by a functional immune system. Preliminary assessment of an antisense IAP therapeutic involves injection of cancer cells infected with the
5 recombinant adenoviruses (sense, antisense, and control viruses) under the skin, and the tumorigenic index compared to that of untreated cells. One may also use this model to assess the effectiveness of systemic administration of antisense oligonucleotides in increasing the efficacy of anti-cancer drugs in the nude mouse model. Phosphorothioate or
10 methylimino substituted oligonucleotides will be assessed at this stage. This type of antisense oligo has demonstrated enhanced cell permeability and slower clearance rates from the body in experimental animal models.

Example 2: Antisense oligonucleotide (ODN) selection

15 We selected 96 or 98, mostly non-overlapping, 19-mer antisense oligonucleotide (ODN) sequences for XIAP and HIAP1, respectively, based on the selection criteria listed below. In the case of XIAP, we selected 96 sequences (each being 19 nucleobases in length) (SEQ ID NOS: 1 through 96; Table 1), from a region approximately 1 kb upstream
20 of the start codon to approximately 1 kb downstream of the stop codon of the cDNA sequence (Fig. 15). This blanketed approximately 50% of the coding region, and immediate 5' and 3' UTR sequences (i.e., 96 19-mers span 1.8 kb of sequence, while the targeted region is approximately 3.5 kb in length, comprised of a coding region of 1.5 kb plus 1 kb at either side of
25 UTR sequences).

Table 1. XIAP Antisense Oligonucleotides

SEQ ID NO:	Code	Position in XIAP Sequence	Antisense Oligonucleotide Sequence
1	A1	2	AAAATTCTAAGTACCTGCA
2	B1	21	TCTAGAGGGTGGCTCAGGA
3	C1	44	CAGATATATATGTAACACT
4	D1	78	TGAGAGCCCTTTTTTTGTT
5	E1	110	AGTATGAAATATTTCTGAT
6	F1	134	ATTGGTTCCAATGTGTTCT
7	G1	160	TTAGCAAAATATGTTTTAA
8	H1	185	TGAATTAATTTTAAATATC
9	A2	238	ATTCAAGGCATCAAAGTTG
10	B2	326	GTCAAATCATTAAATTAGGA
11	C2	370	AATATGTAAACTGTGATGC
12	D2	411	GCAGAATAAACTAATAAT
13	E2	430	GAAAGTAATATTTAAGCAG
14	F2	488	TTACCACATCATTCAAGTC
15	G2	508	CTAAATACTAGAGTTCGAC
16	H2	535	ACACGACCGCTAAGAAACA
17	A3	561	TATCCACTTATGACATAAA
18	B3	580	GTTATAGGAGCTAACAAAT
19	C3	607	AATGTGAAACACAAGCAAC
20	D3	638	ACATTATATTAGGAAATCC
21	E3	653	CTTGTCACCTTTTCTAAA
22	F3	673	ATCTTCTCTTGAAAATAGG
23	G3	694	CCTTCAAACTGTAAAAAG
24	H3	721	ATGTCTGCAGGTACACAAG
25	A4	759	ATCTATTAAACTCTTCTAC

SEQ ID NO:	Code	Position in XIAP Sequence	Antisense Oligonucleotide Sequence
26	B4	796	ACAGGACTACCACTTGGAA
27	C4	815	TGCCAGTGTGATGCTGAA
28	D4	835	GTATAAAGAAACCCTGCTC
29	E4	856	CGCACGGTATCTCCTTCAC
30	F4	882	CTACAGCTGCATGACAACT
31	G4	907	GCTGAGTCTCCATATTGCC
32	H4	930	ATACTTTCCTGTGTCTTCC
33	A5	950	GATAAATCTGCAATTTGGG
34	B5	990	TTGTAGACTGCGTGGCACT
35	C5	1010	ACCATTCTGGATACCAGAA
36	D5	1029	AGTTTTCAACTTTGTACTG
37	E5	1059	ATGATCTCTGCTTCCCAGA
38	F5	1079	AGATGGCCTGTCTAAGGCA
39	G5	1100	AGTTCTCAAAAGATAGTCT
40	H5	1126	GTGTCTGATATATCTACAA
41	A6	1137	TCGGGTATATGGTGTCTGA
42	B6	1146	CAGGGTTCCTCGGGTATAT
43	C6	1165	GCTTCTTCACAATACATGG
44	D6	1192	GGCCAGTCTGAAAGGACT
45	E6	1225	GCTAACTCTCTTGGGGTTA
46	F6	1246	GTGTAGTAGAGTCCAGCAC
47	G6	1273	AAGCACTGCACTTGGTCAC
48	H6	1294	TTCAGTTTCCACCACAAC
49	A7	1316	ACGATCACAAGGTTCCCAA
50	B7	1337	TCGCCTGTGTTCTGACCAG
51	C7	1370	CCGGCCCCAAAACAAAGAAG

SEQ ID NO:	Code	Position in XIAP Sequence	Antisense Oligonucleotide Sequence
52	D7	1393	GATTCAC TTCGAATATTAA
53	E7	1413	TATCAGAACTCACAGCATC
54	F7	1441	GGAAGATTTGTTGAATTTG
55	G7	1462	TCTGCCATGGATGGATTTC
56	H7	1485	AAGTAAAGATCCGTGCTTC
57	A8	1506	CTGAGTATATCCATGTCCC
58	B8	1525	GCAAGCTGCTCCTTGTTAA
59	C8	1546	AAAGCATAAAATCCAGCTC
60	D8	1575	GAAAGCACTTTACTTTATC
61	H8	1610	ACTGGGCTTCCAATCAGTT
62	E8	1629	GTTGTTCCCAAGGGTCTTC
63	F8	1650	ACCCTGGATACCATTTAGC
64	G8	1669	TGTTCTAACAGATATTTGC
65	A9	1688	TATATATTCTTGTCCTTC
66	B9	1696	AGTTAAATGAATATTGTTT
67	C9	1725	GACACTCCTCAAGTGAATG
68	D9	1745	TTTCTCAGTAGTTCTTACC
69	E9	1759	GTTAGTGATGGTGT TTTCT
70	F9	1782	AGATGGTATCATCAATTCT
71	G9	1801	TGTACCATAGGATTTTGGA
72	H9	1820	CCCCATTCTGATAGCTTCT
73	A10	1849	ATTATTTCTTAATGTCCT
74	B10	1893	CAAGTGATTATAGTTGCT
75	C10	1913	TAGATCTGCAACCAGAACC
76	D10	1945	CATCTTGCACTGTCTTT
77	E10	1997	CCTTAGCTGCTCTTCAGTA

SEQ ID NO:	Code	Position in XIAP Sequence	Antisense Oligonucleotide Sequence
78	F10	2018	AAGCTTCTCCTCTTGCAGG
79	G10	2044	ATATTTCTATCCATACAGA
80	H10	2076	CTAGATGTCCACAAGGAAC
81	A11	2096	AGCACATTGTTTACAAGTG
82	B11	2123	AGCACATGGGACACTTGTC
83	C11	2144	CTTGAAAGTAATGACTGTG
84	D11	2182	CCTACTATAGAGTTAGATT
85	E11	2215	ATTCAATCAGGGTAATAAG
86	F11	2234	AAGTCAGTTCACATCACAC
87	G11	2375	CAGTAAAAAAAAATGGATAA
88	H11	2428	TTCAGTTATAGTATGATGC
89	A12	2471	TACACTTAGAAATTAAATC
90	B12	2630	TCTCTATCTTTCCACCAGC
91	C12	2667	AGAATCCTAAAAACAACA
92	D12	2709	ATTCGCACAAGTACGTGTT
93	E12	2785	TGTCAGTACATGTTGGCTC
94	F12	2840	ACATAGTGTTTGGCACTT
95	G12	2861	CTTTGATCTGGCTCAGACT
96	H12	2932	GAAACCACATTTAACAGTT

Note that the three most 5' and the three most 3' nucleobases may comprise DNA residues, or RNA residues, such as 2'-O methyl RNA residues. For example, the antisense oligonucleotide sequence of SEQ ID

5 NO: 3 may be CAGATATATATGTAACACT or CAGATATATATGTAACACU.

A similar approach was taken for designing antisense oligonucleotides against HIAP1. Ninety-eight 19-mer sequences were chosen, with some of the latter sequences picked using less stringent criteria than the originally defined selection criteria (listed below), to increase the number of candidate sequences to study (SEQ ID NOS: 97 through 194; Table 2). Of these 98 sequences targeted to the HIAP1 sequence of Fig. 16, 15 (SEQ ID NOS: 97 through 104, 107, 113, 136, 156, 157, 181, and 193) were selected to evaluate the efficacy of decreasing HIAP1 expression. These 15 candidate sequences consisted of 4 sequences targeting the coding region (SEQ ID NOS: 136, 156, 157, and 181), 1 sequence targeting the 3' UTR (SEQ ID NO: 193), and 7 sequences targeting the 5'UTR (SEQ ID NOS: 100 through 104, 107, and 113; one of the 7 oligonucleotides overlapped the start codon), and 3 other oligonucleotides (SEQ IDs 97 through 99) that were designed to target an intronic segment of the 5'UTR (the value of which is discussed in Example 7). These above-described 15 HIAP1 antisense oligonucleotides were synthesized and tested.

Table 2. HIAP1 Antisense Oligonucleotides

SEQ ID NO	Code	Position in HIAP1 Sequence	Antisense Oligonucleotide Sequence
97	APO 1	1152	TCATTTGAGCCTGGGAGGU
98	APO 2	1172	CGGAGGCTGAGGCAGGAGA
99	APO 3	1207	GGTGTGGTGGTACGCGCCT
100	APO 4	1664	ACCCATGCACAAACTACC
101	APO 5	1865	AGAATGTGCCAGTAGGAGA
102	APO 6	2440	TCTCACAGACGTTGGGCTT
103	APO 7	2469	CCAGTGGTTTGCAAGCATG
104	APO 8	3695	GAAATTTAGTGGCCAGGAA
105		4013	AGAAATACACAATTGCACC
106		4032	TACTGATACATTTTAAGGA
107	APO 9	4057	TTCAACATGGAGATTCTAA
108		4076	ATTTCTATGCATTTAGAGT
109		4121	AATACTAGGCTGAAAAGCC
110		4142	GGCTTTGCTTTTATCAGTT
111		4165	TCTAGGGAGGTAGTTTTGT

SEQ ID NO	Code	Position in HIAP1 Sequence	Antisense Oligonucleotide Sequence
112		4189	GGGAAGAAAAAGGGACTAGC
113	APO 10	4212	GTTCATAATGAAATGAATG
114		4233	ATAAGAATATGCTGTTTC
115		4265	TTCAAACGTGTTGGCGCTT
116		4283	ATGACAAGTCGTATTTTCAG
117		4317	AAGTGGAAACGTAGACAT
118		4338	AGACAGGAACCCAGCAGG
119		4357	CGAGCAAGACTCCTTTCTG
120		4376	AGTGTAATAGAAACCAGCA
121		4395	TGACCTTGTCATTACACC
122		4426	TTATCCAGCATCAGGCCAC
123		4445	ACTGTCTCCTCTTTCCAG
124		4464	TTTATGCTTTTCAGTAGG
125		4489	ACGAATCTGCAGCTAGGAT
126		4517	CAAGTTGTTAACGGAATTT
127		4536	TAGGCTGAGAGGTAGCTTC
128		4555	GTTACTGAAGAAGGAAAAG
129		4574	GAATGAGTGTGTGGAATGT
130		4593	TGTTTTCTGTACCCGGAAG
131		4612	GAGCCACGGAATATCCAC
132		4631	TGATGGAGAGTTTGAATAA
133		4656	GATTTGCTCTGGAGTTAC
134		4670	GGCAGAAAAATCTTGATT
135		4696	GGACAGGGGTAGGAATTC
136	APO 11	4714	GCATTTTCGTTATTCATTG
137		4733	CTGAAAAGTAAGTAATCTG
138		4759	GGCGACAGAAAAGTCAATG
139		4812	CCACTCTGTCTCCAGGTCC
140		4831	CCACCACAGGCAAAGCAAG
141		4855	ITCGGTTCCCAATTGCTCA
142		4874	ITCTGACATAGCATTATCC
143		4893	TGGGAAAATGTCTCAGGTG
144		4907	TATAAATGGGCATTTGGGA
145		4926	TGTCTGAAGCTGATTTTC
146		4945	GAAACTGTGTATCTTGAAG
147		4964	TGTCTGCATGCTCAGATTA
148		4988	GAATGTTTTAAAGCGGGCT
149		5007	CACTAGAGGGCCAGTTAAA
150		5040	CCGCACTTGCAAGCTGCTC
151		5070	CATCATCACTGTTACCCAC
152		5095	CCACCATCACAGCAAAAGC
153		5117	TCCAGATTCCCAACACCTG
154		5130	CCCATGGATCATCTCCAGA
155		5149	AACCACTTGGCATGTTGAA
156	APO 12	5168	CAAGTACTCACACCTTGA
157	APO 13	5187	CCTGTCCTTTAATTCTTAT
158		5206	TGAACTTGACGGATGAACT
159		5225	TAGATGAGGGTAACTGGCT
160		5244	TGGATAGCAGCTGTTCAAG
161		5271	CATTTTCATCTCCTGGGCT

SEQ ID NO	Code	Position in HIAP1 Sequence	Antisense Oligonucleotide Sequence
162		529	TGGATAATTGATGACTCTG
163		5309	GTCTTCTCCAGGTTCAAAA
164		5337	TATTCATCATGATTGCATC
165		5366	CATTTCACGGCAGCATT
166		5367	CCAGGCTTCTACTAAAGCC
167		5416	GCTAGGATTTTCTCTGAA
168		5435	TCTATAATTCTCTCCAGTT
169		5454	ACACAAGATCATTTGACTAG
170		5473	TCTGCATTGAGTAAGTCTA
171		5492	CTCTCCCTTATTTTCATCT
172		5515	TCCTCAGTTGCTCTTCTC
173		5560	GCCATTCTATTCTTCCGGA
174		5579	AGTCAAATGTTGAAAAAGT
175		5598	CCAGGATTGGAATTACACA
176		5622	ATTCCGGCAGTTAGTAGAC
177		5646	TAACATCATGTTCTTGTTTC
178		5675	GTCTGTGTCCTTCTGTTAA
179		5684	TTCTCTTGCTTGTAAGAC
180		5703	CTAAAATCGTATCAATCAG
181	APO 14	5723	GGCTGCAATATTTCTTTT
182		5742	GAGAGTTTCTGAATACAGT
183		5761	ACAGCTTCAGCTTCTTGCA
184		5780	AAATAAATGCTCATATAAC
185		5821	GAAACATCTTCTGTGGGAA
186		5841	GTTCTTCCACTGGTAGATC
187		5862	CTTCTTGAGTCTCCGCAA
188		5890	TTGTCCATACACACTTTAC
189		6097	AACCAAATTAGGATAAAA G
190		6181	ATGTTTCATATGGTTTAGAT
191		6306	TAAGTTTACTTCACTTAC
192		6369	ATGTTCCCGGTATTAGTAC
193	APO 15	6432	GGGCTCAAGTAATTCTCTT
194		6455	GCCCAGGATGGATTCAAAC

Oligonucleotide selection criteria

- The computer program OLIGO (previously distributed by National Biosciences Inc.) was used to define suitable antisense oligonucleotides based on the following criteria: 1) no more than 75% GC content, and no more than 75% AT content; 2) preferably no oligonucleotide with 4 or more consecutive G residues (due to reported toxic effects, although one was chosen as a toxicity control); 3) no oligonucleotides with the ability

to form stable dimers or hairpin structures; and 4) sequences around the translation start site are a preferred region. In addition, accessible regions of the mRNA were predicted with the help of the RNA secondary structure folding program mfold, by M. Zuker (website 1999-2000: <http://mfold2.wustl.edu/~mfold/rna/form1.cgi>). Sub-optimal folds with a free energy value within 5% of the predicted most stable fold of the mRNA were predicted using a window of 200 bases within which a residue can find a complimentary base to form a base pair bond. Open regions that did not form a base pair were summed together with each suboptimal fold and areas that consistently were predicted as open were considered more accessible to the binding of antisense oligonucleotides. Additional oligonucleotides that only partially fulfilled some of the above selection criteria (1-4), were also chosen as possible candidates if they recognized a predicted open region of the target mRNA.

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Example 3: Antisense oligonucleotide synthesis

The antisense oligonucleotides were synthesized by IDT (Integrated DNA Technologies, USA) as chimeric, second-generation oligonucleotides, consisting of a core of phosphodiester DNA residues flanked on either side by two 2'-O methyl RNA residues with a phosphorothioate linkage between the flanking RNA residues. The oligonucleotides were provided in a 96-well plate, as well as matching tubes, with a minimum of 12 ODs of oligo DNA, which provided ample material for transfections (greater than a hundred assays in the 96-well format) when the detection method is a sensitive method, such as TaqMan quantitative PCR, or an ELISA. Once the positive hits were identified (see below), the antisense oligonucleotides were re-synthesized with 3, instead of 2, flanking RNA residues to further increase stability/nuclease resistance. In addition, for validation purposes, appropriate controls (such

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as scrambled, 4-base mismatch, and reverse polarity oligonucleotides) were synthesized for some of the antisense targets that yielded the highest antisense activity.

5 Example 4: Screening assays and optimization of antisense oligonucleotide sequences

Our approach to identifying IAP antisense oligonucleotides was to screen the above-described antisense oligonucleotide libraries for specific decreases (knock-down) of the RNA and protein for the specific IAP gene targeted. Any number of standard assays may be used to detect RNA and protein levels in cells that have been administered an IAP antisense nucleic acid. For example, RNA levels can be measured using standard Northern blot analysis or RT-PCR techniques. In addition, protein levels can be measured, for example, by standard Western blot analyses or immunoprecipitation techniques. Alternatively, cells administered an antisense IAP nucleic acid may be examined for cell viability, according to methods described for example, in U.S. Patent No. 5,919,912, or U.S.S.Ns. 08/576,956, 08/800,929, incorporated herein by reference.

20 We used TaqMan quantitative PCR conditions (described below) to assay for changes in mRNA levels after antisense oligonucleotide treatment, as well as our ELISA method for XIAP and Western blotting (described below) for changes in HIAP1 protein levels, using a polyclonal anti-RIAP1 antibody (rat HIAP1 ortholog; AEgera Therapeutics, Inc.) in the latter case. Transfection conditions were optimized with LipofectAMINE PLUS (Life Technologies, Canada) on T24 bladder carcinoma cells, or lipofectin on SF-295 glioblastoma cells, using a fluorescein-tagged control sense oligo from XIAP spanning the start codon (mGmAG AAG ATG ACT GGT AAmC mA; SEQ ID NO:

195). The results were visualized and gauged by epi-fluorescence microscopy. In addition, in the case of T24 cells, transfections were further optimized based on the ability of a published antisense oligonucleotide to downregulate survivin expression (Li et al., Nat. Cell Biol. 1:461-466, 1999) (U/TGT GCT ATT CTG TGA AU/TU/T SEQ ID NO: 196). We optimized the transfection conditions based on the TaqMan results of survivin RNA knock-down detected with PCR primers and fluorescent probe, described in detail below. Optimal conditions for oligo uptake by the cells were found to be 940 nM oligonucleotide and 40 μ L PLUS reagent and 0.8 μ L LipofectAMINE in a total of 70 μ L for 3 hours. We then applied these conditions to screen for XIAP protein knock-down using the oligo library against T24 cells.

HIAP1 knock-down was studied in SF-295 cells because these cells had easily detectable and discernable 70 kDa HIAP1 protein, while many cell lines do not express high levels of the protein, or are not distinguishable from the large amounts of the similarly sized 68 kDa HIAP2 protein. In fact, there are a number of published errors involving HIAP1 and HIAP2 in the literature because of naming errors in the databases, and because of the poor quality and high crossreactivity, of the various commercial antibodies to HIAP1/cIAP2. The best way to distinguish HIAP1 from HIAP2 is to perform an immunoprecipitation experiment with an IAP antibody (Aegera Therapeutics, Inc.), separate the proteins by 2-dimensional gel electrophoresis, and to then carry out mass spectroscopy analysis of the spots migrating in the 68 to 70 kDa range to verify the identity of the HIAP1 and HIAP2 bands, using standard methods known in the art. This method determines if HIAP1 and HIAP2 co-migrate at the 68 kDa position, and if the 70 kDa form of HIAP1 results from a splice variant or a post-translational modification.

Real-time PCR

RNA was extracted from cells lysed in RLT buffer (QIAGEN, Inc., Canada), and purified using QIAGEN RNeasy columns/kits.

Real-time quantitative PCR was performed on a Perkin-Elmer ABI 7700

5 Prism PCR machine. RNA was reverse transcribed and amplified according to the TaqMan Universal PCR Master Mix protocol of PE Biosystems, using primers and probes designed to specifically recognize XIAP, HIAP1, survivin, or GAPDH. For human survivin, the forward primer was 5'-TCT GCT TCA AGG AGC TGG AA-3', the reverse primer

10 was 5'-GAA AGG AAA GCG CAA CCG-3', and the probe was 5'-(FAM) AGC CAG ATG ACG ACC CCA TAG AGG AAC ATA(TAMRA)-3' (SEQ ID NOS: 197 through 199). For human HIAP1, the forward primer was 5'-TGG AGA TGA TCC ATG GGT TCA-3', the reverse primer was 5'-GAA CTC CTG TCC TTT AAT TCT TAT CAA

15 GT-3', and the probe was 5'-(FAM) CTC ACA CCT TGG AAA CCA CTT GGC ATG(TAMRA)-3' (SEQ ID NOS: 200 through 202). For human XIAP, the forward primer was 5'-GGT GAT AAA GTA AAG TGC TTT CAC TGT-3', the reverse primer was 5'-TCA GTA GTT CTT ACC AGA CAC TCC TCA A-3', and the probe was 5'-(FAM) CAA CAT

20 GCT AAA TGG TAT CCA GGG TGC AAA TAT C(TAMRA)-3' (SEQ ID NOS: 203 through 205). For human GAPDH, the forward primer was 5'-GAA GGT GAA GGT CGG AGT C-3', the reverse primer was 5'-GAA GAT GGT GAT GGG ATT C-3', and the probe was 5'-(JOE) CAA GCT TCC CGT TCT CAG CC(TAMRA)-3' (SEQ ID NOS: 206 through

25 208).

Relative quantitation of gene expression was performed as described in the PE Biosystems manual using GAPDH as an internal standard. The comparative Ct (cycle threshold) method was used for relative quantitation of IAP mRNA levels compared to GAPDH mRNA

levels. Briefly, real-time fluorescence measurements were taken at each PCR cycle and the threshold cycle (Ct) value for each sample was calculated by determining the point at which fluorescence exceeded a threshold limit of 30 times the baseline standard deviation. The average
5 baseline value and the baseline SD are calculated starting from the third cycle baseline value and stopping at the baseline value three cycles before the signal starts to exponentially rise. The PCR primers and/or probes for the specific IAPs were designed to span at least one exon-intron boundary separated by 1 or more kb of genomic DNA, to reduce the possibility of
10 amplifying and detecting genomic DNA contamination. The specificity of the signal, and possible contamination from DNA, were verified by treating some RNA samples with either DNase or RNase, prior to performing the reverse transcription and PCR reaction steps.

15 *XIAP ELISA and HIAP1 Western immunoblots*

A standard colorimetric XIAP ELISA assay was performed using an affinity-purified rabbit polyclonal antibody to XIAP (Aegera Therapeutics, Inc.) as a capture antibody, and was detected with a XIAP monoclonal antibody (MBL, Japan) and a biotinylated anti-mouse Ig
20 antibody and horseradish peroxidase-conjugated streptavidin and TMB substrate. Alternatively, a polyclonal XIAP or HIAP1 antibody may be used to measure XIAP or HIAP1 protein levels, respectively.

HIAP1 was detected on a Western immunoblot using an affinity-purified anti-HIAP1 rabbit polyclonal antibody as a primary
25 antibody and was detected by ECL (Amersham) on X-ray film with a secondary horseradish-peroxidase-conjugated anti-rabbit Ig antibody and a chemiluminescent substrate. The anti-HIAP1 polyclonal antibody is raised against a GST-fusion of the rat ortholog of HIAP1. This antibody cross-reacts with both human and murine HIAP1 and HIAP2.

Example 5: Antisense XIAP oligonucleotides decrease XIAP RNA and polypeptide expression

The XIAP synthetic library of 96 antisense oligonucleotides was first screened for decreases in XIAP protein levels. Specifically, T24 cells (1.5 x 10⁴ cells/well) were seeded in wells of a 96-well plate on day 1, and were cultured in antibiotic-free McCoy's medium for 24 hours. On day 2, the cells were transfected with XIAP antisense oligonucleotides as described above (oligonucleotides are labeled according to their plated position, i.e., A1 to H12, and include 2 repeats, A13 and B13 that contain lyophilized DNA pellets that stuck to the sealing membrane). Briefly, the oligos were diluted in 10 µl/well of serum-free, antibiotic-free McCoy's medium and then PLUS reagent was added. LipofectAMINE was diluted in 10 µl/well of serum-free, antibiotic-free McCoy's medium, and both mixes were incubated for 15 minutes at room temperature. The mixes were then combined and incubated for 15 minutes at room temperature.

In the meantime, the complete medium was removed from the cells and 50 µl/well of serum-free, antibiotic-free medium was added to the cells. The transfection mixes were added to the well, and the cells were incubated for 3 hours. Then 30 µl/well of serum-free, antibiotic-free medium and 100 µl/well of antibiotic-free complete medium, containing 2X fetal bovine serum were added to each well.

At day 3, XIAP RNA levels were measured using quantitative real-time PCR techniques, as described above. At day 4, XIAP protein levels were measured by ELISA (Figs. 7A, 7C, 7E, 7G, 7I, and 7K), and total cellular protein was measured biochemically (Figs. 7B, 7D, 7F, 7H, 7J, and 7L; used to normalize the XIAP protein levels). The results were compared to a mock transfection sample (treated with the transfection agent but no oligonucleotide DNA was added, and then processed as for

the other samples). Time course experiments determined that the optimal time for protein knock-down to be around 12 to 24 hours.

The library was also screened for decreases in RNA levels, using TaqMan- specific PCR primers and fluorescent probes at the appropriate
5 optimal time, using the primers and probes described above. Time course experiments determined mRNA to be optimally decreased at 6 to 9 hours. These results agree well with the protein results.

The first screen (although performed at a sub-optimal time point when XIAP levels are returning to normal, possibly due to an outgrowth
10 of non-transfected cells) identified 16 antisense oligonucleotides (ODNs C2, E2, E3, F3, C4, D4, E4, F4, G4, C5, D5, B6, F6, D7, D8, F8) out of the total 96 antisense oligonucleotides tested that showed some decrease in XIAP protein levels relative to total protein, compared to mock (no ODN) transfection levels (Fig. 7A, 7C, 7E, 7G, 7I, and 7K). Interestingly, total
15 protein was decreased for each of these 16 ODNs, which indicates a toxic or cytostatic effect of these ODNs (Fig. 7B, 7D, 7F, 7H, 7J, 7L). Note that ODNs B9 and C9 showed a clear drop in total protein but no relative drop in XIAP protein levels. These 16 hits were then validated more rigorously at more optimal time points XIAP protein and RNA knock-down results at
20 12 hours after the start of transfection.

The 16 antisense ODNs that showed some decrease in relative XIAP protein levels compared to mock transfection, were re-tested alone or in combination, with one control oligo (D2) included, for their ability to knock-down XIAP protein at a more optimal time point (12 hours) based
25 on the above described time course studies (Fig. 8B). these ODNs were also examined for their ability to decrease XIAP mRNA levels at 12 hours, normalized against GAPDH levels, and compared to mock transfection. Total protein concentrations at 12 hours were also determined (Fig. 8C).

There was a good correlation between the ability of an antisense ODN to decrease XIAP protein levels (Fig. 8B) with its ability to decrease XIAP mRNA levels (Fig. 8A). In addition, there is no major loss of total protein at this early time point, and the decrease in XIAP mRNA and protein precede the decrease in total protein that is seen at later time points. The ODNs that showed greater than 50% loss of XIAP protein or mRNA levels alone, or in a combination of two ODNs added at a 0.5:0.5 ratio, were identified as the best ODNs and validated further. Of these 16 oligonucleotides, 10 of them (ODNs E2, E3, F3, E4, F4, G4, C5, B6, D7, F8) showed a consistent ability to decrease XIAP protein or RNA levels by more than 50%, depending on the transfection conditions used, or when used in combination, as for ODNs C5 and G4.

Interestingly, these 16 oligonucleotides that demonstrated antisense activity clustered in 4 different target regions of the XIAP mRNA, with adjacent ODNs showing some knock-down activity. No antisense activity was observed by oligonucleotides that target sequences between these regions or islands of sensitivity. Presumably, these regions represent open areas on the mRNA that are accessible to antisense ODNs inside the cell. Two antisense oligonucleotides, E3 and F3, target XIAP just upstream of the start codon in the intervening region between the IRES and the translation start site, and partially overlap the end of the IRES element. ODNs C2, D2, and E2 target a XIAP region upstream of the minimal IRES element, providing further evidence that the minimal IRES region is a highly structured region of RNA which is not readily accessible to antisense ODNs *in vivo*. All the other antisense ODN hits fall within the coding region, including a cluster of activity at positions 856-916 of the XIAP sequence of Fig. 15 (ODNs E4, F4, and G4) and smaller separate areas, as demonstrated by ODNs C5 and D5, for example.

Example 6: XIAP antisense oligonucleotides increase cytotoxicity and chemosensitization

We also investigated if XIAP antisense ODNs could chemosensitize the highly drug resistant T24 cells to traditional
5 chemotherapeutic drugs, such as adriamycin or cisplatin. Antisense ODNs were chosen to represent some of the different XIAP target regions and were tested for their cytotoxic effects, alone or in combination with other ODNs or drugs. Five of the ten best XIAP antisense oligonucleotides were tested for their ability to kill or chemosensitize T24 bladder
10 carcinoma cells, and were compared to the effects of three corresponding scrambled control ODNs.

T24 cells were transfected with XIAP antisense oligonucleotides, scrambled oligonucleotides, no oligonucleotides (mock transfected), or were left untreated. The cells were tested for viability 20 hours after
15 transfection (with the exception of the untreated control) using the WST-1 tetrazolium dye assay in which WST-1 tetrazolium dye is reduced to a colored formazan product in metabolically active cells (Fig 9A). Alternatively, cell viability is tested using any one of the above described apoptosis methods.

20 The occurrence of cytotoxicity induced by the antisense XIAP ODN E4 was examined by visually inspecting T24 cells that were left untreated, mock transfected, or transfected with E4 antisense ODNs, E4 scrambled ODNs, E4 reverse polarity, or E4 mismatched ODNs. Twenty hours after transfection, the cells were examined for morphology (Fig. 9D). Only the
25 cell transfected with antisense E4 ODNs showed signs of toxicity.

To examine the effects of the oligonucleotides on the chemosensitization of the T24 cells to cisplatin or adriamycin, oligonucleotides were tested for their ability to further kill T24 cells in the presence of a fixed dose of adriamycin (0.5 µg/ml). Cells were first

transfected with the oligonucleotides, then adriamycin was added for another 20 hours. Viability was measured by WST-1 at the end of the 20 hour drug treatment (Fig. 9B). Values are shown as percentage viability compared to their ODN treatment alone results shown in Fig. 9C. Fig. 9C is essentially a repeat of Fig. 9A, but with the actual corresponding values used in calculating the results for the chemosensitization experiment in Fig. 9B.

All 5 oligonucleotides tested (ODNs F3, E4, G4, C5, D7, or the combinations of E4+C5, or G4+C5) killed the T24 cells, leaving only 10-15% surviving cells after 24 hours, as compared to the mock (no ODN) transfected cells, or to cells transfected with 3 corresponding scrambled controls to F3 (mCmAmG AGA TTT CAT TTA AmCmG mU; SEQ ID NO: 209), E4 (mCmUmA CgC TCg CCA TCg TmUmC mA; SEQ ID NO: 210) and C5 (mUmGmC CCA AGA ATA CTA GmUmC mA; SEQ ID NO: 211)(Figs. 9A and C). Therefore, the toxicity is sequence-specific to those ODNs that reduce XIAP levels, and not to a non-sequence specific toxicity due to ODNs of this chemistry in general, as three scrambled controls did not show any more toxicity compared to the mock transfected control. This cytotoxicity may result from the combined effect of XIAP protein knock-down (and the expected loss of anti-apoptotic protection afforded by XIAP) and the cytotoxicity of the transfection itself. Both mock (no ODN) and scrambled ODN transfections resulted in an approximately 40% decrease in survival as compared to untreated cells (Fig. 9A). This is not unexpected, as the opposite is true (i.e., overexpression of IAPs protect insect cells from cytofectin-mediated cell death, a liposomal transfection agent similar to the ones used in these studies (Jones et al., J. Biol. Chem. 275:22157-22165, 2000)

The addition of a fixed dose of adriamycin or cisplatin at the end of the 3 hour transfection period resulted in a further decrease in survival for

some of the tested oligonucleotides, a further 40% drop in survival after 20 hours for ODNs F3, D7 and G4+C5 combination (Fig. 9B), compared to their corresponding ODNs treated values (Fig. 9C). Note that the values in Fig. 9B (ODN plus drug) are compared to their corresponding ODN survival (ODN alone) in Fig. 9C, which is set a 100% for each ODN. Only the results for adriamycin chemosensitization are shown; however, similar results were obtained when the cells were chemosensitized with cisplatin. At the fixed doses used, the mock and scrambled control transfections did not show any increased loss of survival when either treated with adriamycin (Fig. 9B). Chemosensitization is only seen when XIAP levels are decreased by a specific antisense ODN.

Example 7: Antisense HIAP1 oligonucleotides decrease HIAP1 RNA and polypeptide expression

The smaller library of 15 HIAP1 antisense oligonucleotides was screened for protein knock-down by Western and for RNA knock-down by TaqMan, using the primers and probes described above, under two different conditions. Alternatively, HIAP1 RNA levels may be detected using standard Northern blot analyses or RT-PCR techniques. The antisense oligonucleotides were administered to cells under basal conditions or under cycloheximide-induction conditions (24 hour treatment with sub-toxic doses). We have discovered that cycloheximide (CHX) can lead to a 10- to 200-fold induction of HIAP1 mRNA depending on the cell line treated. This in turn leads to an increase in HIAP1 protein, as seen on a Western blot (70 kDa band). We have also discovered that this effect of CHX is via two distinct mechanisms of action. First, CHX activates NFkB, a known transcriptional inducer of HIAP1, by blocking the *de novo* synthesis of a labile protein, IkB, which

is an inhibitor of NFkB. This effect is mimicked by puromycin, another protein synthesis inhibitor, and by TNF-alpha, which induces a signaling cascade leading to the phosphorylation, ubiquitination, and degradation of Ikb. However, only CHX leads to a further stabilization of the HIAP1 mRNA, as seen by the decreased rate of disappearance of HIAP1 message in the presence of actinomycin D, to block *de novo* transcription, and CHX, as opposed to actinomycin D and puromycin or TNF-alpha combined.

SF295 glioblastoma cells were transfected with lipofectin and ODN (scrambled survivin, no oligo or mock, antisense APO1 to APO15) or left untreated. RNA was isolated from the cells 6 hours after transfection and the level of HIAP1 mRNA was measured by quantitative PCR (TaqMan analysis), normalized for GAPDH mRNA, with the value for the scrambled survivin ODN transfection set as 1.0.

The results of this experiment, a compilation of three separate experiments, are shown in Fig. 10. The scrambled survivin ODN, the mock transfection, and untreated (non-transfected) cells, all showed similar HIAP1 mRNA levels. Of the 15 antisense ODNs, 7 oligonucleotides (ODNs APO 1, -2, -7, -8, -9, -12, -15) showed an almost 50% decrease when compared to mock transfection or survivin scrambled control (mUmAmA GCT GTT CTA TGT GmUmU mC; SEQ ID NO: 212) ODN transfection (Fig. 10). Some of the ODNs led to an induction in HIAP1 mRNA, which may be a stress response to a non-specific toxic ODN. The antisense ODN, however, may still be effective at knocking down HIAP1 protein levels even if the message is increased if the ODN is able to interfere with the translation process.

The effect of HIAP1 antisense oligonucleotides on HIAP1 protein and mRNA expression was also examined in cells induced to express HIAP1. SF295 cells were transfected with ODNs, or were mock

transfected. The transfected cells were then treated with 10 µg/ml cycloheximide for 24 hours to induce 70 kDa HIAP1 mRNA and protein. Protein levels were measured by Western immunoblot analysis with an anti-HIAP1 polyclonal antibody, and normalized against actin protein in a re-probing of the same blots. Scans of the Western blot results are shown in Fig. 11A. The densitometric scan results were plotted against the mock results (set at 100%) in Fig. 11B. A line is drawn at 50% to easily identify the most effective antisense ODNs. The transfection process itself (e.g., mock or scrambled survivin) induces HIAP1 protein compared to the untreated sample as shown on the Western immunoblot.

Of the 15 tested ODNs, 6 of them (APO 1, -2, -7, -8, -12, and -15) showed the strongest activity, or had significant activity in both the protein and mRNA assays, and did not cause a stress-induced increase in HIAP1 mRNA, such as that seen with ODNs APO 4, -6, -11, -13, -14 (Fig. 10), and by control ODNs to APO 2 (mismatch or reverse polarity, see text below and Figs. 12 and 13). Note that APO 6 also showed evidence of toxicity as seen by the general decrease in total protein (Fig. 12).

To further investigate the efficacy of HIAP1 antisense oligonucleotides under cycloheximide induction conditions, changes in HIAP1 mRNA were measured by TaqMan real time PCR 6 hours after transfection with ODN APO 2, which targets an Alu repeat within an intron of HIAP1 and results in the greatest block of CHX-induced upregulation of HIAP1 mRNA and protein. Controls for this experiment were three ODNs for APO 2: one scrambled sequence (same base composition but random order, AAG GGC GGC GGA GTG AGA C; SEQ ID NO: 213), one reverse polarity (same base composition, same sequential order but in the opposite direction, AGA GGA CGG AGT CGG

AGG C; SEQ ID NO: 214), and one mismatch sequence (containing 4 base mismatches out of 19 bases, CGG AGC GTG AGG ATG GAG A; SEQ ID NO: 215).

Transfection of the APO 2 antisense into cells resulted in a 50% decrease in mRNA compared to a scrambled survivin control and matched perfectly with the protein results, while the scrambled control for APO 2 (H1 sc apo 2 in Fig. 13) did not change HIAP1 mRNA levels at all (repeated twice here, and in two different experiments). However, the mismatch control ODN (H1 mm apo 2) and the reverse polarity control ODN (H1 RV apo 2) showed an induction of 6 to 7 fold in HIAP1 mRNA at 6 hours. These ODNs no longer targeted HIAP1, as expected, but may still target Alu repeats because of the degeneracy and repeat nature of these sequences. Therefore, it is possible that these two controls are toxic to the cell and cause a stress response that leads to the induction of HIAP1. This effect may also occur with the antisense APO 2 ODN, but in this case, the APO 2 ODN also causes the degradation of the induced HIAP1 mRNA which results in a relative decrease of HIAP1 mRNA, compared to a scrambled survivin control, as well as decreasing the relative fold induction of HIAP1 protein after transfection and CHX treatment, compared to scrambled survivin control ODN.

The 6 optimal antisense HIAP1 ODNs include two very effective antisense ODNs against an intronic sequence (APO 1, and -2; with APO 2 demonstrating the best activity). These oligonucleotides have some interesting properties that could be of great use therapeutically for cancer or autoimmune disorders. The oligonucleotides against an intronic sequence would likely only target pre-mRNA (very short-lived target) and not the mature, processed form of HIAP1. Typically, introns are not targeted for antisense except when one wants to alter splicing by targeting the intron-exon boundaries or the branching point. These usually result in

the skipping of an exon rather than RNase-mediated degradation of the message. Both mechanisms would likely be favorable for the enhancement of apoptosis, as the skipping would result in the loss of the exon encoding the first two important BIR domains of HIAP1. The APO-
5 2 antisense oligo also targets an intron of survivin for 18 consecutive bases out of 19, but we did not see any loss of survivin protein; only HIAP1 was decreased after the oligo treatment, demonstrating the specificity of the HIAP1 antisense oligonucleotide. These antisense oligonucleotides hit Alu sequences in the HIAP1 intron and potentially in many other genes,
10 and induce the cancer cells to die (see below), which may be as a result of down regulating HIAP1 and some other critical genes, and thus of therapeutic value if it is not too toxic to normal cells.

Cancer cells have reportedly more Alu-containing transcripts and may therefore be more sensitive to apoptosis induction with an Alu
15 targeting antisense ODN. Furthermore, this killing effect of APO 1 and APO 2 ODNs may be due to the combined effect of both targeting Alu sequences and HIAP1 simultaneously. This dual effect would result in an effective way to prevent the normal stress response of HIAP1 induction through the NFkB pathway, when the cell is exposed to certain toxic
20 agents. This stress response is most likely part of the cancer cell's anti-apoptotic program. By blocking HIAP1 expression, we counter this anti-apoptotic stress response and precipitate the cancer cell's demise.

Example 8: HIAP1 antisense oligonucleotides increase cytotoxicity and
25 chemosensitization

The effect of HIAP1 antisense oligonucleotides on the chemosentization of SF295 cells was also evaluated. Cells were transfected with one of 3 different antisense ODNs (APO 7, APO 15, and Scrambled APO 2 (control)). Twenty-four hours after tranfection with the

ODNs, the cells were incubated with adriamycin for an additional 24 hours before assaying by for cell survival by assaying WST-1.

The WST-1 survival curves for SF295 cells transfected with the above-described HIAP1 ODNs and then treated with increasing concentrations of adriamycin are shown in Fig. 14. The two ODNs that resulted in a decrease in HIAP1 mRNA also showed a decrease in survival when treated with adriamycin compared to cells treated with an ODN which did not reduce HIAP1 mRNA levels. Therefore, reducing HIAP1 levels by antisense, or other means, can chemosensitize a glioblastoma cell line that is highly resistant to the cytotoxic action of many chemotherapeutic drugs.

Example 9: *In vivo* analyses of IAP antisense oligonucleotides

Antisense oligonucleotides that decrease expression of IAP in cell culture models can be tested in animals. For example, the antisense oligonucleotide can be tested in mice according to the method of Lopes de Menezes et al. (Clin. Cancer Res. 6: 2891-2902, 2000) or Klasa et al. (Clin. Cancer Res. 6: 2492-2500, 2000). Antisense and control ODNs are tested, for example, in sub-cutaneous human xenografts of breast cancer, colon cancer, lung cancer, squamous cell carcinoma or prostate cancer in SCID mice. The antisense oligonucleotides are also tested in an orthotopic model for the prostate, as well as in a disseminated non-Hodgkin's lymphoma model. The mouse's tolerance to cisplatin, taxol, doxorubicin, and cyclophosphamide is known for each of these models.

In vivo testing of the antisense oligonucleotides involves intraperitoneal injections (once a day, on days 3 through 7, 10 through 14, and 17 through 21) of naked ODN of (5 mg/kg), with or without a chemotherapeutic drug. Alternatively, liposomal type carriers for the ODNs may also be employed. Oligos are injected shortly after tumors

cells have been seeded in the mouse, or when the tumor has established and grown to a size of 0.1-0.15 g. Tumor size is then monitored to determine if the ODN treatments or ODN plus drug treatments reduce the growth rate of the tumor, lead to regression, or have no effect at all. In
5 another alternative, ODNs in liposomal formulation are injected directly into the tumors.

Example 10: Anti-IAP antibodies

In order to generate IAP-specific antibodies, an IAP coding
10 sequence (e.g., amino acids 180-276) can be expressed as a C-terminal fusion with glutathione S-transferase (GST; Smith et al., Gene 67:31-40, 1988). The fusion protein can be purified on glutathione-Sepharose beads, eluted with glutathione, and cleaved with thrombin (at the engineered cleavage site), and purified to the degree required to successfully
15 immunize rabbits. Primary immunizations can be carried out with Freund's complete adjuvant and subsequent immunizations performed with Freund's incomplete adjuvant. Antibody titres are monitored by Western blot and immunoprecipitation analyses using the thrombin-cleaved IAP fragment of the GST-IAP fusion protein. Immune sera are
20 affinity-purified using CNBr-Sepharose-coupled IAP protein. Antiserum specificity is determined using a panel of unrelated GST proteins (including GSTp53, Rb, HPV-16 E6, and E6-AP) and GST-trypsin (which was generated by PCR using known sequences).

As an alternate or adjunct immunogen to GST fusion proteins,
25 peptides corresponding to relatively unique hydrophilic regions of IAP may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity-purified on peptides conjugated to BSA, and specificity is tested by ELISA and Western blotting, using peptide

conjugates, and by Western blotting and immunoprecipitation using IAP expressed as a GST fusion protein.

Alternatively, monoclonal antibodies may be prepared using the IAP proteins described above and standard hybridoma technology (see, e.g., Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, New York, NY, 1981; Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 1994). Once produced, monoclonal antibodies are also tested for specific IAP recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., *supra*).

Antibodies that specifically recognize IAPs or fragments of IAPs, such as those described in U.S.S.N. 08/800,929, incorporated herein by reference, containing one or more BIR domains (but not a ring zinc finger domain), or that contain a ring zinc finger domain (but not a BIR domain) are considered useful in the invention. They may, for example, be used in an immunoassay to monitor IAP expression levels or to determine the subcellular location of an IAP or IAP fragment produced by a mammal. Antibodies that inhibit the 26 kDa IAP cleavage product described herein (which contains at least one BIR domain) may be especially useful in inducing apoptosis in cells undergoing undesirable proliferation.

Preferably antibodies of the invention are produced using IAP sequence that does not reside within highly conserved regions, and that appears likely to be antigenic, as analyzed by criteria such as those provided by the Peptide structure program (Genetics Computer Group Sequence Analysis Package, Program Manual for the GCG Package, Version 7, 1991) using the algorithm of Jameson and Wolf (CABIOS 4:181, 1988). Specifically, these regions, which are found between BIR1

and BIR2 of all IAPs, are: from amino acid 99 to amino acid 170 of HIAP1, from amino acid 123 to amino acid 184 of HIAP2, and from amino acid 116 to amino acid 133 of either XIAP or m-XIAP. These fragments can be generated by standard techniques, e.g., by the PCR, and
5 cloned into the pGEX expression vector (Ausubel et al., *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel et al. (*supra*). In order to minimize the potential for obtaining antisera that is non-specific, or exhibits low-affinity binding to IAP, two or three fusions are generated for each
10 protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in series, preferably including at least three booster injections.

Example 11: Comparison of cell survival following transfection with full
15 length vs. partial IAP constructs

In order to investigate the mechanism whereby human IAPs, including XIAP, HIAP1, and HIAP2, afford protection against cell death, expression vectors were constructed that contained either: (1) full-length IAP cDNA (as described in U.S.S.N. 08/800,929), (2) a portion of an IAP
20 gene that encodes the BIR domains, but not the RZF, or (3) a portion of an IAP gene that encodes the RZF, but not the BIR domains. Human and murine XIAP cDNAs were tested by transient or stable expression in HeLa, Jurkat, and CHO cell lines. Following transfection, apoptosis was induced by serum withdrawal, application of menadione, or application of
25 an anti-Fas antibody. Cell death was then assessed by trypan blue exclusion. As a control for transfection efficiency, the cells were co-transfected with a Beta-gal expression construct. Typically, approximately 20% of the cells were successfully transfected.

When CHO cells were transiently transfected, constructs containing full-length human or mouse XIAP cDNAs conferred modest but definite protection against cell death. In contrast, the survival of CHO cells transfected with constructs encoding only the BIR domains (i.e., lacking the RZF domain) was markedly enhanced 72 hours after serum deprivation. Furthermore, a large percentage of cells expressing the BIR domains were still viable after 96 hours, at which time no viable cells remained in the control, i.e. non-transfected, cell cultures, and less than 5% of the cells transfected with the vector only, i.e., lacking a cDNA insert, remained viable. Deletion of any of the BIR domains results in the complete loss of apoptotic suppression, which is reflected by a decrease in the percentage of surviving CHO cells to control levels within 72 hours of serum withdrawal.

Stable pools of transfected CHO cells, which were maintained for several months under G418 selection, were induced to undergo apoptosis by exposure to 10 μ M menadione for 2 hours. Among the CHO cells tested were those that were stably transfected with: (1) full-length murine XIAP cDNA (miap), (2) full-length XIAP cDNA (XIAP), (3) full-length bcl-2 cDNA (Bcl-2), (4) cDNA encoding the three BIR domains (but not the RZF) of murine XIAP (BIR), and (5) cDNA encoding the RZF (but not BIR domains) of m-XIAP (RZF). Cells that were non-transfected (CHO) or transfected with the vector only (pcDNA3), served as controls for this experiment. Following exposure to 10 μ M menadione, the transfected cells were washed with phosphate buffered saline (PBS) and cultured for an additional 24 hours in menadione-free medium. Cell death was assessed, as described above, by trypan blue exclusion. Less than 10% of the non-transfected or vector-only transfected cells remained viable at the end of the 24 hour survival period. Cells expressing the RZF did not fare significantly better. However, expression of full-length

murine XIAP, human XIAP, or bcl-2, and expression of the BIR domains, enhanced cell survival. When the concentration of menadione was increased from 10 μ M to 20 μ M (with all other conditions of the experiment being the same as when 10 μ M menadione was applied), the percentage of viable CHO cells that expressed the BIR domain cDNA
5 construct was higher than the percentage of viable cells that expressed either full-length murine XIAP or bcl-2.

Example 12: Analysis of the subcellular location of expressed RZF and

10 BIR domains

The assays of cell death described above indicate that the RZF acts as a negative regulator of the anti-apoptotic function of IAPs. One way in which the RZF, and possibly other IAP domains, may exert their regulatory influence is by altering the expression of genes, whose products
15 function in the apoptotic pathway.

In order to determine whether the subcellular locations of expressed RZF and BIR domains are consistent with roles as nuclear regulatory factors, COS cells were transiently transfected with the following four constructs, and the expressed polypeptide was localized by
20 immunofluorescent microscopy: (1) pcDNA3-6myc-xiap, which encodes all 497 amino acids of SEQ ID NO:219, (2) pcDNA3-6myc-m-xiap, which encodes all 497 amino acids of mouse XIAP (SEQ ID NO:225), (3) pcDNA3-6myc-mxiap-BIR, which encodes amino acids 1 to 341 of m-xiap (SEQ ID NO:225), and (4) pcDNA3-6myc-mxiap-RZF, which
25 encodes amino acids 342-497 of murine xiap (SEQ ID NO:225). The cells were grown on multi-well tissue culture slides for 12 hours, and then fixed and permeabilized with methanol. The constructs used (here and in the cell death assays) were tagged with a human Myc epitope tag at the N-terminus. Therefore, a monoclonal anti-Myc antibody and a secondary

goat anti-mouse antibody, which was conjugated to FITC, could be used to localize the expressed products in transiently transfected COS cells. Full-length XIAP and MIAP were located in the cytoplasm, with accentuated expression in the peri-nuclear zone. The same pattern of localization was observed when the cells expressed a construct encoding the RZF domain (but not the BIR domains). However, cells expressing the BIR domains (without the RZF) exhibited, primarily, nuclear staining. The protein expressed by the BIR domain construct appeared to be in various stages of transfer to the nucleus.

10

Other Embodiments

All publications and patent applications mentioned in this specification, including U.S. Patent No. 5,919,912 and U.S.S.Ns. 08/576,956 and 08/800,929 are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

15

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

20

25

Claims

1. An inhibitor of apoptosis (IAP) antisense nucleic acid that
inhibits IAP biological activity, regardless of length of said antisense
5 nucleic acid.
2. The antisense IAP nucleic acid of claim 1, wherein said IAP is
XIAP.
- 10 3. The antisense IAP nucleic acid of claim 1, wherein said IAP is
HIAP1.
4. The antisense IAP nucleic acid of claim 1, wherein said IAP is
HIAP2.
15
5. The antisense IAP nucleic acid of claim 1, wherein said
antisense nucleic acid is mammalian.
6. The antisense IAP nucleic acid of claim 5, wherein said
20 antisense nucleic acid is human.
7. The antisense nucleic acid of claim 1, wherein said antisense
nucleic acid is between 8 and 30 nucleotides in length.
- 25 8. The antisense IAP nucleic acid of claim 2, wherein said
antisense is chosen from any one of SEQ ID NOS: 1 through 96.
9. The antisense IAP nucleic acid of claim 3, wherein said
antisense is chosen from any one of SEQ ID NOS: 97 through 194.

10. The antisense IAP nucleic acid of claim 1, wherein said IAP biological activity is inhibition of apoptosis.

11. The antisense IAP nucleic acid of claim 1, wherein said IAP
5 biological activity is inhibition of IAP polypeptide expression.

12. The antisense IAP nucleic acid of claim 1, wherein said antisense nucleic acid comprises at least one modified internucleoside linkage.

10

13. The antisense IAP nucleic acid of claim 12, wherein said modified internucleoside linkage is selected from the group consisting of phosphorothioate, methylphosphonate, phosphotriester, phosphorodithioate, and phosphoselenate linkages.

15

14. The antisense IAP nucleic acid of claim 1, wherein said antisense nucleic acid comprises at least one modified sugar moiety.

15. The antisense IAP nucleic acid of claim 14, wherein said
20 modified sugar moiety is a 2'-O methyl group.

16. The antisense IAP nucleic acid of claim 1, wherein said antisense nucleic acid is a chimeric nucleic acid.

25 17. The antisense IAP nucleic acid of claim 16, wherein said chimeric nucleic acid comprises DNA residues linked together by phosphorothioate linkages, said DNA residues flanked on each side by at least one 2'-O methyl RNA residues linked together by phosphorothioate linkages.

18. The antisense IAP nucleic acid of claim 17, wherein said DNA residues are flanked on each side by at least 3 2'-O methyl RNA residues.

19. The antisense nucleic acid of claim 1, wherein said antisense
5 nucleic acid is a ribozyme.

20. A method of enhancing apoptosis in a cell, said method comprising administering a negative regulator of the IAP anti-apoptotic pathway to said cell.

10 21. The method of claim 20, wherein said negative regulator is an antisense IAP nucleic acid.

22. The method of claim 21, wherein said IAP is XIAP.

15 23. The method of claim 21, wherein said IAP is HIAP1.

24. The method of claim 21, wherein said IAP is HIAP2.

20 25. The method of claim 21, wherein said antisense nucleic acid is mammalian.

26 The method of claim 25, wherein said antisense nucleic acid is human.

25 27. The method of claim 22, wherein said antisense is chosen from any one of SEQ ID NOS: 1 through 96.

28. The method of claim 23, wherein said antisense is chosen from any one of SEQ ID NOS: 97 through 194.

29. The method of claim 21, wherein said antisense nucleic acid
5 comprises at least one modified internucleoside linkage.

30. The method of claim 29, wherein said modified internucleoside linkage is selected from the group consisting of phosphorothioate, methylphosphonate, phosphotriester, phosphorodithioate, and
10 phosphoselenate linkages.

31. The method of claim 21, wherein said antisense nucleic acid comprises at least one modified sugar moiety.

15 32. The method of claim 31, wherein said modified sugar moiety is a 2'-O methyl group.

33. The method of claim 21, wherein said antisense nucleic acid is a chimeric nucleic acid.
20

34. The method of claim 33, wherein said chimeric nucleic acid comprises DNA residues linked together by phosphorothioate linkages, said DNA residues flanked on each side by at least one 2'-O methyl RNA residues linked together by phosphorothioate linkages.
25

35. The method of claim 34, wherein said DNA residues are flanked on each side by at least 3 2'-O methyl RNA residues.

36. The method of claim 21, wherein said administration sensitizes said cell to chemotherapy.

37. The method of claim 21, wherein said administration sensitizes
5 said cell to radiotherapy.

38. The method of claim 20, wherein said negative regulator is an antibody that specifically binds an IAP polypeptide.

10 39. The method of claim 20, wherein said negative regulator is an IAP polypeptide comprising a ring zinc finger, said polypeptide having no more than two BIR domains.

40. The method of claim 20, wherein said negative regulator is a
15 nucleic acid encoding the ring zinc finger domain of an IAP polypeptide.

41. The method of claim 20, wherein said negative regulator is a compound that prevents cleavage of the IAP polypeptide.

20 42. The method of claim 20, wherein said cell is *in vitro*.

43. The method of claim 20, wherein said cell is *in vivo*.

44. The method of claim 43, wherein said cell is in a mammal
25 diagnosed with a proliferative disease.

45. A pharmaceutical composition comprising a mammalian IAP antisense nucleic acid.

46. The pharmaceutical composition of claim 45, wherein said antisense nucleic acid binds a target sequence of the human XIAP gene or mRNA.

5 47. The pharmaceutical composition of claim 45, wherein said antisense nucleic acid binds a target sequence of the human HIAP1 gene or mRNA.

10 48. The pharmaceutical composition of claim 45, wherein said antisense nucleic acid binds a target sequence of the human HIAP2 gene or mRNA.

15 49. The pharmaceutical composition of claim 45, wherein said antisense nucleic acid binds a target sequence of the murine XIAP gene or mRNA.

20 50. The pharmaceutical composition of claim 45, wherein said antisense nucleic acid binds a target sequence of the murine HIAP1 gene or mRNA.

25 51. The pharmaceutical composition of claim 45, wherein said antisense nucleic acid binds a target sequence of the murine HIAP2 gene or mRNA.

52. The pharmaceutical composition of claim 45, wherein said mammalian antisense IAP nucleic acid is human antisense nucleic acid.

53. The pharmaceutical composition of claim 46, wherein said antisense is chosen from any one of SEQ ID NOS: 1 through 96.

54. The pharmaceutical composition of claim 47, wherein said antisense is chosen from any one of SEQ ID NOS: 97 through 194.

FIG. 1A

HUMAN xiap

1 gaaaagggtggacaagtcctaatttcaagagaagatgacttttaacagttttgaaggatctt + 60
 a M T F N S F E G S -
 61 aaaacttggtacctgcagacatcaataagggaagaattttagaagagtttaataga + 120
 a K T C V P A D I N K E E F V E E F N R -
 121 ttaaaaacttttgctaattttccaagtggtagtcctgtttcagcatcaacactggcacga + 180
 a L K T F A N F P S G S P V S A S T L A R -
 181 gcagggtttctttatactggtgaaggagataccgtgcgggtgcttttagttgctcatgcagct + 240
 a A G F L Y T G E G D T V R C F S C H A A -
 241 gtatatagatggcaatatggagactcagcagttggaagacacaggaagtatccccaaat + 300
 a V D R W Q Y G D S A V G R H R K V S P N -
 301 tgcagatttatcaacggcttttatcttgaaaaatagtgccacgcagctctacaaaattcttgggt + 360
 a C R F I N G F Y L E N S A T Q S T N S G -

1/67

2/67

FIG. 1B

HUMAN xiap

```

361  atccagaatggtcagtagacaagaagttgaaaaactatctgggaagcagagatcatcttgcctta 420
      I Q N G Q Y K V E N Y L G S R D H F A L -
421  gacaggccatctgagacacatgcagactatcttttgagaactgggcagggttagatatata 480
      D R P S E T H A D Y L L R T G Q V V D I -
481  tcagacaccatataccgaggaaccctgccatgtattgtgaagaagctagattaaagtcc 540
      S D T I Y P R N P A M Y C E E A R L K S -
541  ttccagaactggccagactatgctcacctaaccccaagagagttagcaagtgcaggactc 600
      F Q N W P D Y A H L T P R E L A S A G L -
601  tactacacagggtattggtgaccaagtgagtgcttttgtgtggtggaactgaaaaat 660
      Y Y T G I G D Q V Q C F C C G G K L K N -
661  tgggaaccttgtgatcgtgcctggtcagaacacacaggcgacactttcctaattgcttcttc 720
      W E P C D R A W S E H R R R H F P N C F F -

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3/67

FIG. 1C

HUMAN xiap

```

a      gttttggccggaatcttaatatctgaagtgaatctgatgctgtgagttctgataggaat 780
      +-----+-----+-----+-----+-----+-----+
      V L G R N L N I R S E S D A V S S D R N -
      +-----+-----+-----+-----+-----+-----+
      ttcccaaatccaacaaatcttccaagaaatcccatccatggcagattatgaagcacggatc 840
      +-----+-----+-----+-----+-----+-----+
      F P N S T N L P R N P S M A D Y E A R I -
      +-----+-----+-----+-----+-----+-----+
      ttacttttgggacatggatatactcagttaacaaggagcagcttgcaagagctggattt 900
      +-----+-----+-----+-----+-----+-----+
      F T F G T W I Y S V N K E Q L A R A G F -
      +-----+-----+-----+-----+-----+-----+
      tatgctttaggtgaaggatgataaaagtgaagtgtcttctcactgtggaggaggctaaactgat 960
      +-----+-----+-----+-----+-----+-----+
      Y A L G E G D K V K C F H C G G G L T D -
      +-----+-----+-----+-----+-----+-----+
      tggaagcccagtgagacccttgggaacaacatgctaaatgggtatccagggtgcaaatat 1020
      +-----+-----+-----+-----+-----+-----+
      W K P S E D P W E Q H A K W Y P G C K Y -
      +-----+-----+-----+-----+-----+-----+
      ctgttagaacagaaggacaagaatatataaacaatatcttaactcattcacttgag 1080
      +-----+-----+-----+-----+-----+-----+
      L L E Q K G Q E Y I N N I H L T H S L E -

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4/67

FIG. 1D

HUMAN xiap

```

1081  gagtgctgtaagaactactgagaaaaacaccatcactaactagaagaattgatgatacc 1140
      E C L V R T T E K T P S L T R R I D D T -
1141  atcttccaaaatcctatggtacaagaagctatacgaatgggggttcagttttcaaggacatt 1200
      I F Q N P M V Q E A I R M G F S F K D I -
1201  aagaaaaataatggaggaaaaaattcagatatctcgggagcaactataaatacacttgaggtt 1260
      K K I M E E K I Q I S G S N Y K S L E V -
1261  ctggttgcagatctagtgaatgctcagaaagacagtatgcaagatgagtcagtcagact 1320
      L V A D L V N A Q K D S M Q D E S S Q T -
1321  tcattacagaaagagatttagtactgaagagcagctaaggcgctgcaagaggagaagctt 1380
      S L Q K E I S T E E Q L R R L Q E E K L -
1381  tgcaaaatctgtatgtagaataattgctatcgtttttctccttctgtggacatctagtc 1440
      C K I C M D R N I A I V F V P C G H L V -

```


FIG. 1E

HUMAN xiap

1441 acttgaacaatgtgctgaagcagttgacaagtgtcccatgtgctacacagtcattact + 1500
 a T C K Q C A E A V D K C P M C Y T V I T -
 1501 ttcaagcaaaaaattttatgtcttaacttaactctatagtaggcattgttattgtttct + 1560
 a F K Q K I F M S * -
 1561 tattaccctgattgaatgtgtgatgtgaactgactttaagtaatcaggattgaattccat + 1620
 a -
 1621 tagcatttgctaccaagtaggaaaaaaatgtacatggcagtgtttttagttggcaatata + 1680
 a -
 1681 atccttgaaatttcttgatttttcagggtattagctgtattatccatttttttactgtta + 1740
 a -
 1741 ttttaattgaaaccatagactaagaataagaagcatcatactataactgaacacaatgtgt + 1800
 a -

5/67

6/67

FIG. 1F

HUMAN xiap

1801 attcatagtatactgatttaatttctaagtgttaagtgaattaatcatctggatcttttat 1860
a -

1861 tcttttcagataggcttaacaaatggagcttctgtatatataaaatgtggagattagagtta 1920
a -

1921 atctcccgaatcacataatttgtttgtgtgaaaaaggaataaaattgttccatgctggtg 1980
a -

1981 gaaagatagagattgttttttagagggtggttgtgtgttttaggattctgtccattttct 2040
a -

2041 tgtaaaagnnataaacacgnacntgtgcgaaatatntttgtaaagtgatcttgccattnttg 2100
a -

2101 aaagcgtattttaatgatagaatactatcgcagccaaacatgtactgacatggaaagatgtca 2160
a -

7/67

FIG. 1G HUMAN xiap

```

2161  nagatatgttaagtgtataaatgcaagtggcnnnacactatgtatagtctgagccagatca 2220
      -----+-----+-----+-----+-----+
a      -

2221  aagtatgtatgttnttaatatgcatagaacnanagatttgaaagatatataccaaactg 2280
      -----+-----+-----+-----+-----+
a      -

2281  ttaaattgtgtttctcttcggggagggggggattgggggagggggccagaggggttta 2340
      -----+-----+-----+-----+-----+
a      -

2341  naggggccttttcactttcnacttttttcattttgttctgttcgnattttttataagtat 2400
      -----+-----+-----+-----+-----+
a      -

2401  gtanacccnaagggttttatggnaactaacatcagtaacctaacccccgtgactatcct 2460
      -----+-----+-----+-----+-----+
a      -

2461  gtncctcttcctaggagctgtntgtttccaccaccacccttccctctgaacaaatgc 2520
      -----+-----+-----+-----+-----+
a      -

2521  ctgagtgcctggggcactttn 2540
      -----+-----+
a      -

```

8/67

FIG. 2A

HUMAN hiap-1

```

      TCCTTGAGATGTATCAGTATAGGATTTAGGATCTCCATGTTGGAACTCTAAATGCATAGA
1  -----+-----+-----+-----+-----+-----+-----+-----+ 60
      -
      AATGGAAATAATGGAAATTTTTCATTTTGGCTTTTCAGCCCTAGTATTAAAACTGATAAAA
61 -----+-----+-----+-----+-----+-----+-----+-----+ 120
      -
      GCAAGCCATGCACAAAACCTACCTCCCTAGAGAAAGGCTAGTCCCTTTCTTCCCCATTC
121 -----+-----+-----+-----+-----+-----+-----+-----+ 180
      -
      ATTTCATATGACATAGTAGAAAACAGCATATTTCTTATCAAAATTTGATGAAAAGCGCCA
181 -----+-----+-----+-----+-----+-----+-----+-----+ 240
      M N I V E N S I F L S N L M K S A N -
      ACACGTTTGAACCTGAAAATACGACTTGTCAATGTGAACCTGTACCGAATGCTACGTATTCCA
241 -----+-----+-----+-----+-----+-----+-----+-----+ 300
      T F E L K Y D L S C E L Y R M S T Y S T -
      CTTTTCCTGCTGGGGTTCCTGTCTCAGAAAGGAGTCTTGCTCGTGGTTTCTATTACA
301 -----+-----+-----+-----+-----+-----+-----+-----+ 360
      F P A G V P V S E R S L A R A G F Y Y T -
      C
```

9/67

FIG. 2B

HUMAN hiap-1

```

361  CTGGTGTGAATGACAAGGTCAAATGCTTCTGTGTGGCCTGATGCTGGATAACTGGAAAA 420
      G V N D K V K C C F C C G L M L D N W K R -

421  GAGGAGACAGTCCTACTGAAAGCATAAAAAGTTGTATCCTAGCTGCAGATTCGTTTCAGA 480
      G D S P T E K H K K L Y P S C R F V Q S -

481  GTCTAAATTCGTTAACTGGAAGCTACCTCTCAGCCCTACTTTTCCTTCTTCAGTAA 540
      L N S V N N L E A T S Q P T F P S S V T -

541  CACATTCCACACACTCATTTACTTCCGGGTACAGAAACAGTGGATATTTCCGTGGCTCTT 600
      H S T H S L L P G T E N S G Y F R G S Y -

601  ATTCAAACTCTCCATCAAATCCTGTAACTCCAGAGCAAAATCAAGAATTTCTGCCTTGA 660
      S N S P S N P V N S R A N Q E F S A L M -

661  TGAGAAGTTCCTACCCCTGTCCAATGAATAACGAAATGCCAGATTACTTACTTTTCAGA 720
      R S S Y P C P M N N E N A R L L T F Q T -

```

10/67

FIG. 2C

HUMAN hiap-1

```

721  CATGGCCATTGACTTTTCTGTCGCCAACAGATCTGGCACGACGAGCTTTTACTACATAG 780
      W P L T F L S P T D L A R A G F Y Y I G -

781  GACCTGGAGACAGAGTGGCTTGCTTTGCCCTGTGGTGGAATAATTGAGCAATTGGGAACCGA 840
      P G D R V A C F A C G G K L S N W E P K -

841  AGGATAATGCTATGTCAGAAACACCTGAGACATTTTCCCAAATGCCCATTTATAGAAAATC 900
      D N A M S E H L R H F P K C P F I E N Q -

901  AGCTTCAAGACACTTCAAGATACACAGTTTCTAATCTGAGCATGCAGACACATGCAGCCCC 960
      L Q D T S R Y T V S N L S M Q T H A A R -

961  GCTTTAAACATTCTTTAACTGGCCCTCTAGTGTTCTAGTTAATCCTGAGCAGCTTGCAA 1020
      F K T F F N W P S S V L V N P E Q L A S -

1021 GTGCGGGTTTTTATTATGTGGGTAAACAGTGATGATGTCAAATGCTTTTGTGTGATGGTG 1080
      A G F Y Y V G N S D D V K C F C C D G G -

```

11/67

FIG. 2D

HUMAN hiap-1

```

1081  GACTCAGGTGTTGGGAATCTGGAGATGATCCATGGGTTCACATGCCAAGTGGTTTCCAA 1140
      L R C W E S G D D P W V Q H A K W F P R -

1141  GGTGTCAGTACTTGATAAGAATTAAAGGACAGGAGTTCATCCGTCAAGTTCGAAGCCAGTT 1200
      C E Y L I R I K G Q E F I R Q V Q A S Y -

1201  ACCCTCATCTACTTGAACAGCTGCTATCCACATCAGACAGCCAGGAGATGAAATGCAG 1260
      P H L L E Q L L S T S D S P G D E N A E -

1261  AGTCATCAATTATCCATTGGAACCTGGAGAAGACCATTTCAGAGATGCAATCATGATGA 1320
      S S I I H L E P G E D H S E D A I M M N -

1321  ATACTCCTGTGATTAAATGCTGCCGTGGAAATGGGCTTTAGTAGAAGCCTGGTAAACAGA 1380
      T P V I N A A V E M G F S R S L V K Q T -

1381  CAGTTCAGAGAAAAATCCTAGCAACTGGAGAGAAATTATAGACTAGTCAATGATCTTGTGT 1440
      V Q R K I L A T G E N Y R L V N D L V L -

```

12/67

FIG. 2E

HUMAN hiap-1

```

1441 TAGACTTACTCAATGCAGAAGATGAAATAAGGGAAGAGGAGAGAGAAAGAGCAACTGAGG 1500
      D L L N A E D E I R E E E R A T E E -
1501 AAAAAGAATCAATGATTTATTATTATCCGGAAGAATAGAAATGGCACTTTTCAACATT 1560
      K E S N D L L L I R K N R M A L F Q H L -
1561 TGACTTGTGTAATCCAATCCTGGATAGTCTACTAACTGCCGGAATTATTAAATGAACAAG 1620
      T C V I P I L D S L L T A G I I N E Q E -
1621 AACATGATGTTATTAAACAGAAGACACAGACGCTCTTACAAGCAAGAACTGATGATA 1680
      H D V I K Q K T Q T S L Q A R E L I D T -
1681 CGATTTAGTAAAGGAAATATTGCAGCCACTGTATTCAGAAACTCTCTGCAAGAAGCTG 1740
      I L V K G N I A A T V F R N S L Q E A E -
1741 AAGCTGTGTATATGAGCATTATTGTGCAACAGGACATATAATATATCCACAGAAG 1800
      A V L Y E H L F V Q Q D I K Y I P T E D -

```


13/67

FIG. 2F

HUMAN hiap-1

1801 ATGTTTCAGATCTACCAGTGAAGAACAATTGCGGAGACTACCAGAAGAAACATGTA 1860
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
C V S D L P V E E Q L R R L P E E R T C K -

1861 AAGTGTGTATGGACAAAGAAGTGTCCTATAGTGTTATTCCCTTGTGGTCATCTAGTAGTAT 1920
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
C V C M D K E V S I V F I P C G H L V V C -

1921 GCAAAGATTGTGCTCCTTCTTTAAGAAAGTGTCCTATTTGTAGGAGTACAATCAAGGGTA 1980
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
C K D C A P S L R K C P I C R S T I K G T -

1981 CAGTTCGTACATTCTTTTCATGAAGAAGAACCAACATCGTCTAAACTTTAGAAATTAAT 2040
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
C V R T F L S * -

2041 TTATTAAATGTATTATAACTTTAACTTTTATCCCTAATTTGGTTTCCTTAAATTTTATT 2100
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
C TATTACAAC TCAAAAACATTGTTTGTGTAAACATATTATATATGTATCTAAACCATA -

2101 2160
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
C -

FIG. 2G

HUMAN hiap-1

2161 TGAACATATATTTTGTAGAACTAAGAGAAATGATAGGCTTTTGTCTTATGAACGAAAA 2220

2221 GAGGTAGCACTACAAACACAATATTCAATCCAAATTTTCAGCATTATTGAAATTGTAAGTG
2280

2281 AAGTAAACTTAAGATATTGAGTTAACCTTTAAGAAATTTTAAATATTTGGCATTGTAC 2340

2341 TAATACCGGGAACATGAAGCCAGGTGTGGTGTATGTACCTGTAGTCCCAGGCTGAGGCA 2400

AGAGAAATTACTTGAGCCCGAGGAGTTTGAATCCATCCTGGGCAGCATACTGAGACCCCTGCC
2401 -----+-----+-----+-----+-----+-----+-----+ 2460

2461 TTTAAACXAAACAGXACCAAXCCAACACCAGGACACATTTCTCTGTCTTTTGTGAT 2520

15/67

FIG. 2H

HUMAN hiap-1

2521 CAGTGTCTATACATCGAAGGTGTGCATATATGTTGAATCACATTTTAGGACATGGTGT 2580

2581 TTTTATAAAGAATTCTGTGAGXAAAAAATTTAATAAGCAACCCXAAATTACTCTTAAAAAA 2640

2641 AAAAAAAAAAAAAAATCGAGGGCCCGTACCAAT 2676

C C C

HUMAN hiap-2

SUBSTITUTE SHEET (RULE 26)

17/67

FIG. 3B

HUMAN hiap-2

361	GACTTTTCCTGTGAACTCTACAGAAATGCTACATAATTCAACTTTCCTCCGCCGGGTGCCT	420
a	D F S C E L Y R M S T Y S T F P A G V P	-
421	GTCTCAGAAAGAGTCTTGCTCGTCTGGTGTATATATACTGGTGTGAATGACAAAGTC	480
a	V S E R S L A R A G F Y Y T G V N D K V	-
481	AAATGCTTCTGTGTGGCCTGATGCTGGATAAAGTGAAGTGAAGACAGTCCTATTCAA	540
a	K C F C C G L M L D N W K L G D S P I Q	-
541	AAGCATAAACAGCTATATCCTAGCTGTAGCTTTATTCAGAATCTGGTTTCAGCTAGTCTG	600
a	K H K Q L Y P S C S F I Q N L V S A S L	-
601	GGATCCACCTCTAAGAATACGTCCTCCAATGAGAAACAGTTTGCACATTTCATTCTCCC	660
a	G S T S K N T S P M R N S F A H S L S P	-
661	ACCTTGGAACATAGTCTGTTCAGTGGTCTTACTCCAGCCTTCCTCCAAACCCCTCTT	720
a	T L E H S S L F S G S Y S S L P P N P L	-

18/67

FIG. 3C

HUMAN hiap-2

```

a      AATTCTAGAGCAGTTGAAGACATCTCTTCATCGAGGACTAACCCCTACAGTTATGCAATG      721
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      N S R A V E D I S S S R T N P Y S Y A M -
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      AGTACTGAAGAAGCCAGATTCTTACCTACCATATGTGGCCATTAACTTTTTTGTCAACCA      781
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      S T E E A R F L T Y H M W P L T F L S P -
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      TCAGAAATTGGCAAGAGCTGGTTTTTATTATATAGGACCTGGAGATAGGGTAGCCTGCTTT      841
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      S E L A R A G F Y Y I G P G D R V A C F -
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      GCCGTGTGGGAAGCTCAGTAACTGGGAACCAAGGATGATGCTATGTCAGAACACCCGG      901
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      A C G G K L S N W E P K D D A M S E H R -
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      AGGCATTTCCCAACTGTCCATTTTGGAAAATCTCTAGAAACTCTGAGGTTTAGCATT      961
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      R H F P N C P F L E N S L E T L R F S I -
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      TCAAATCTGAGCATGCAGACACATGCAGCTCGAATGAGAACATTTATGTACTGGCCATCT      1021
-----+-----+-----+-----+-----+-----+-----+-----+-----+
a      S N L S M Q T H A A R M R T F M Y W P S -
-----+-----+-----+-----+-----+-----+-----+-----+-----+

```

19/67

FIG. 3D

HUMAN hiap-2

```

1081  AGTGTTCAGTTCAGCCTGAGCAGCTTGCAAGTGCTGGTTTATTATGTGGGTCGCAAT 1140
      S V P V Q P E Q L A S A G F Y Y V G R N -
1141  GATGATGTCAAATGCTTGGTTGTGATGGTGGCTTGAGGTGTGGGAATCTGGAGATGAT 1200
      D D V K C F G C D G G L R C W E S G D D -
1201  CCATGGGTAGAACATGCCAAGTGGTTCCAAAGGTGTGAGTCTCTGATACGAATGAAAGGC 1260
      P W V E H A K W F P R C E F L I R M K G -
1261  CAAGAGTTTGTGATGAGATTCAAGGTAGATATCCTCATCTTCTTGAACAGCTGTGTCA 1320
      Q E F V D E I Q G R Y P H L L E Q L L S -
1321  ACTTCAGATACCACCTGGAGAAGAAATGCTGACCCACCAATTATTCATTTTGGACCTGGA 1380
      T S D T T G E E N A D P P I I H F G P G -
1381  GAAAGTCTTCAGAAAGATGCTGTGTCATGATGAATACACCTGTGGTTAAATCTGCCTTGGA 1440
      E S S S E D A V M M N T P V V K S A L E -

```

20/67

FIG. 3E

HUMAN hiap-2

a	1441	ATGGGCTTTAATAGAGACCTGGTGAAACAAACAGTTCTTAAGTAAATCCTGACAACTGGA	1500
		M G F N R D L V K Q T V L S K I L T T G -	
	1501	GAGAACTATAAAACAGTTAATGATATGTGTCAGCACTTCTTAATGCTGAAGATGAAAAA	1560
a		E N Y K T V N D I V S A L L N A E D E K -	
	1561	AGAGAAGAGGAGAGGAAACAAAGCTGAAGAAATGGCATCAGATGATTGTGCAATTAAT	1620
a		R E E E K E K Q A E E M A S D D L S L I -	
	1621	CGGAAGAACAGAAATGGCTCTCTTTCAACAATTGACATGTGTGCTTCCTATCCTGGATAAT	1680
a		R K N R M A L F Q Q L T C V L P I L D N -	
	1681	CTTTTAAAGGCCAATGTAATTAATAAACAGGAACATGATATATTAAACAAAAACACAG	1740
a		L L K A N V I N K Q E H D I I K Q K T Q -	
	1741	ATACCTTTACAGCGAGAGAACTGATTGATACCATTGGGTTAAGGAAATGCTGCGGCC	1800
a		I P L Q A R E L I D T I W V K G N A A A -	

FIG. 3F

HUMAN hiap-2

1801 AACATCTTCAAAAGCTGTCTAAAGAAATTGACTCTACATTGTATAAGAACTTATTGTG 1860
a N I F K N C L K E I D S T L Y K N L F V -
1861 GATAAGAAATATGAAGTATATCCACAGAAGATGTTTCAGGCTGTGCTCACTGGAAGAACAA 1920
a D K N M K Y I P T E D V S G L S L E E Q -
1921 TTGAGGAGGTTGCAAGAAGAACGAACTTGTAAGTGTGTATGGACAAAGTTTCTGT 1980
a L R R L Q E E R T C K V C M D K E V S V -
1981 GTATTATTCCCTTGTGGTCATCTGGTAGTATGCCAGGAATGTGCCCCCTTCTCTAAGAAA 2040
a V F I P C G H L V V C Q E C A P S L R K -
2041 TGCCCTATTTCAGGGGTATAATCAAGGGTACTGTTCGTACATTCTCTTAAAGAAA 2100
a C P I C R G I I K G T V R T F L S -
2101 ATAGTCTATATTTAACCTGCATAAAAGGTCTTTAAATATTTGTTGAACACTTGAAGCC 2160
a -

21/67

HUMAN hiap-2

2161	ATCTAAAGTAAAAAGGGAATTATGAGTTTTTCAATTAGTAACATTCATGTTCTAGTCTGC	2220
a	-	-
2221	TTTGGTACTAATAATCTTGTCTGAAAAGATGGTATCATATATTTAAATCTTAATCTGTGT	2280
a	-	-
2281	TATTACAAGGGAAGATTTATGTTTGGTGAACATATATTAGTATGTATGTACCTAAGGG	2340
a	-	-
2341	AGTAGCGTCXCTGCTTGTTATGCATCATTTTCAGGAGTTACTGGATTGTGTTGTTCTTTCAG	2400
a	-	-
2401	AAAGCTTTGAAXACTAAATTATAGTGTAGAAAAGAACTGGAAACCAGGAACCTCTGGAGTT	2460
a	-	-
2461	CATCAGAGTTATGGTGCCGAATTGTCTTTGGTGCTTTTTCACCTTGTTGTTTAAATAAGGA	2520
a	-	-
2521	TTTTTCTCTTATTTCTCCCCCTAGTTTGTGAGAAACATCTCAATAAAGTGCTTTAAAAAG	2580
a	-	-

FIG. 4A

MOUSE xiap

1 G A C A C T C T G C T G G G C G G C G C C C T C C T C C G G A C C T C C C C T C G G A A C C G T C G C C C + 60
 -
 61 G C G C G C C T T A G T T A G G A C T G G A G T G C T T G G C G C G A A A A G G T G G A C A A G T C C T A T T T C C A + 120
 -
 121 G A G A A G A T G A C T T T T A A C A G T T T T G A A G G A A C T A G A A C T T T T G T A C T T G C A G A C A C C A A T + 180
 -
 a M T F N S F E G T R T F V L A D T N -
 181 A A G G A T G A A G A A T T T G T A G A A G A G T T T A A T A G A T T A A A A C A T T T G C T A A C T T C C C A A G T + 240
 -
 a K D E E F V E E F N R L K T F A N F P S -
 241 A G T A G T C C T G T T T C A G C A T C A A C A T T G G C G C G A G C T G G G T T C T T T A T A C C G G T G A A G G A + 300
 -
 a S S P V S A S T L A R A G F L Y T G E G -
 301 G A C A C C G T G C A A T G T T T C A G T T G T C A T G C G G C A A T A G A T A G A T G C G A G T A T G G A G A C T C A + 360
 -
 a D T V Q C F S C H A A I D R W Q Y G D S -

23/67

FIG. 4B

MOUSE xiap

24/67

```

361      GCTGTTGGAAGACACAGGAGAATATCCCAAAATTGCAGATTATCAATGGTTTATTTT      420
      -----+-----+-----+-----+-----+-----+-----+-----+
a      A V G R H R R I S P N C R F I N G F Y F      -

421      GAAAATGGTGCTGCACAGTCTACAAATCCTGGTATCCAAATGGCCAGTACAAATCTGAA      480
      -----+-----+-----+-----+-----+-----+-----+-----+
a      E N G A A Q S T N P G I Q N G Q Y K S E      -

481      AACTGTGTGGAAATAGAAATCCTTTGGCCCTGACAGGCCACCTGAGACTCATGCTGAT      540
      -----+-----+-----+-----+-----+-----+-----+-----+
a      N C V G N R N P F A P D R P P E T H A D      -

541      TATCTCTGAGAACTGGACAGGTTGTAGATATTTCAGACACCATATACCCGAGGAACCCCT      600
      -----+-----+-----+-----+-----+-----+-----+-----+
a      Y L L R T G Q V V D I S D T I Y P R N P      -

601      GCCATGTGTAGTGAAGAAGCCAGATTGAAGTCATTTCAGAACTGGCCGGACTATGCTCAT      660
      -----+-----+-----+-----+-----+-----+-----+-----+
a      A M C S E E A R L K S F Q N W P D Y A H      -

661      TTAACCCCCAGAGAGTTAGCTAGTGTGGCCTCTACTACACAGGGGCTGATGATCAAGTG      720
      -----+-----+-----+-----+-----+-----+-----+-----+
a      L T P R E L A S A G L Y Y T G A D D Q V      -

```

25/67

FIG. 4C

MOUSE xiap

CAATGCTTTTGTGTGGGAAAACTGAAAAAATTGGGAACCCCTGTGATCGTGCCTGGTCA
 -----+-----+-----+-----+-----+-----+-----+-----+ 780

a Q C C C C G G K L K N W E P C D R A W S -
GAACACAGGAGACACTTCCCAATTGCTTTTGTGGCCCGAACGTTAATGTTCTCGA
781 -----+-----+-----+-----+-----+-----+ 840

[illegible]

a S E S G V S' S D R N F P N S T N S P R N -
CCAGCCATGGCAGAATATGAAGCACGGATCGTTACTTTGGAAACATGGATATACTCAGTT
901 -----+-----+-----+-----+-----+-----+ 960

a P A M A E Y E A R I V T F G T W I Y S V -
AACAAGGAGCAGCTTGCAAGAGCTGGATTTTATGCTTTAGGTGAAGCGGATAAAGTGAAG
961 -----+-----+-----+-----+-----+-----+ 1020

a N K E Q L A R A G F Y A L G E G D K V K -
TGCTTCCACTGTGGAGGAGGGCTCAGGATTTGGAAGCCAAAGTGAAGACCCCTGGGACCAG
1021 -----+-----+-----+-----+-----+ 1080

a C F H C G G L T D W K P S E D P W D Q -

26/67

FIG. 4D

MOUSE xiap

```

1081  CATGCTAAGTGCTACCCAGGGTGCAAATACCTATTGGATGAGAAGGGCAAGATATATA 1140
      H A K C Y P G C K Y L L D E K G Q E Y I -
1141  AATAATATTCATTAAACCCATCCACTTGAGGAATCTTTGGGAAGAACTGCTGAAAAACA 1200
      N N I H L T H P L E E S L G R T A E K T -
1201  CCACCGCTAACTAAAAATCGATGATACCAATCTCCAGAATCCTATGGTGCAAGAAGCT 1260
      P P L T K K I D D T I F Q N P M V Q E A -
1261  ATACGAATGGGATTAGCTTCAAGGACCTTAAGAAAACAATGGAAGAAAAATCCAAACA 1320
      I R M G F S F K D L K K T M E E K I Q T -
1321  TCCGGGAGCAGCTATCTACTTGAGGTCCTGATTCAGATCTTGTGAGTGCAGAAA 1380
      S G S S Y L S L E V L I A D L V S A Q K -
1381  GATAACGGAGGATGAGTCAAGTCAAACTTCATTGCAGAAAGACATTAGTACTGAAGAG 1440
      D N T E D E S S Q T S L Q K D I S T E E -

```

27/67

FIG. 4E

MOUSE xiap

```

1441 CAGCTAAGGCGCCTACAAGAGGAGAAGCTTCCAAAATCTGTATGGATAGAAATATTGCT
-----+-----+-----+-----+-----+-----+-----+-----+
a Q L R R L Q E E K L S K I C M D R N I A -
1501 ATCGTTTTTTCCTTGTGGACATCTGGCCACTTGTAAACAGTGTGCAGAACGAGTTGAC
-----+-----+-----+-----+-----+-----+-----+-----+
a I V F F P C G H L A T C K Q C A E A V D -
1561 AAATGTCCCATGTGCTACACCGTCATTACGTTCAACCAAAAATTTTATGTCTTAGTGG
-----+-----+-----+-----+-----+-----+-----+-----+
a K C P M C Y T V I T F N Q K I F M S * -
1621 GGCACCACATGTTATGTTCTTCTTGCTCTAATTGAATGTGTAATGGAGCGGAACCTTTAAG
-----+-----+-----+-----+-----+-----+-----+-----+
a -
1681 TAATCCTGCAATTGCATTCATAGCATCCCTGCTGTTCCTCAATGGAGACCAATGCTAAC
-----+-----+-----+-----+-----+-----+-----+-----+
a -
1741 AGCACTGTTTCCGTCTAAACATTCAATTTCTGGATCTTTTCGAGTTATCAGCTGTATCAT
-----+-----+-----+-----+-----+-----+-----+-----+
a -
1800

```

28/67

FIG. 4F

MOUSE xiap

```
1801 TAGCCAGTGTTTACTCGATTGAACCTTAGACAGAGAGCAATTTATAGCTTTTCACAT 1860
a
1861 GTATATTGGTAGTACACTGACTTGATTCTCTATATGTAAGTGAATTCATCACCTGCCATGTT 1920
a
1921 TCATGCCCTTTTGCATAAGCTTAACAAATGGAGTGTTCTGTATAAGCATGGAGATGTGATG 1980
a
1981 GAATCTGCCCAAATGACTTTAATTGGCTTATTGTAAACACCGAAAGAACTGCCCCACGCTG 2040
a
2041 CTGGGAGGATAAAGATTGTTTATAGATGCTCACTTCTGTGTTTATAGGATCTGCCCCATTTA 2100
```


29/67

FIG. 5A

M-hiap-1

```

GAATTCCGGAGACCTACACCCCGGAGATCAGAGGTCAATGCTGGCGTTCAGAGCCTAG
1  -----+-----+-----+-----+-----+-----+-----+
GAAGTGGGCTGCGGTATCAGCCTAGCAGTAAACCGACCAGAGCCATGCACAAACTAC
61 -----+-----+-----+-----+-----+-----+-----+
ATCCCAGAGAAAGACTTGTCCTTCCCTCCCTGTCATCTCACCATGAACATGGTTCAA
121 -----+-----+-----+-----+-----+-----+-----+
                                     M N M V Q -
a

GACAGCGCCTTCTAGCCAAGCTGAAGAAGTGCTGACACCTTTGAGTTGAAGTATGAC
181 -----+-----+-----+-----+-----+-----+-----+
D S A F L A K L M K S A D T F E L K Y D -
a

TTTTCCCTGTGAGCTGTACCGATTGTCCACGTATTCAGCTTTTCCAGGGGAGTTCCTGTG
241 -----+-----+-----+-----+-----+-----+-----+
F S C E L Y R L S T Y S A F P R G V P V -
a

TCAGAAAGGAGTCTGGCTCGTGGCTTTTACTACACTGGTGCCCAATGACAAGGTCAAG
301 -----+-----+-----+-----+-----+-----+-----+
S E R S L A R A G F Y Y T G A N D K V K -
a

TGCTTCTGCTGTGGCCTGATGCTAGACAACTGGAACAAGGGACAGTCCCATGGAGAAG
361 -----+-----+-----+-----+-----+-----+-----+
C F C C G L M L D N W K Q G D S P M E K -
a

```

30/67

FIG. 5B

M-hiap-1

```

CACAGAAAGTTGTACCCAGCTGCAACTTTGTACAGACTTTGAATCCAGCCAACAGTCTG
421 -----+-----+-----+-----+-----+ 480
a H R K L Y P S C N F V Q T L N P A N S L -

GAAGCTAGTCCTCGGCCTTCTCTCCACGGCGATGAGCACCATGCCTTTGAGCTTT
481 -----+-----+-----+-----+-----+ 540
a E A S P R P S L P S T A M S T M P L S F -

GCAAGTTCTGAGAATACTGGCTATTTCAAGTGGCTTTACTCGAGCTTTCCCTCAGACCTT
541 -----+-----+-----+-----+-----+ 600
a A S S E N T G Y F S G S Y S S F P S D P -

GTGAAC TTCCGAGCAAATCAAGATTGTCCTGCTTTGAGCACAAAGTCCCTACCAC TTGCA
601 -----+-----+-----+-----+-----+ 660
a V N F R A N Q D C P A L S T S P Y H F A -

ATGAACACAGAGAAGGCCAGATTACTCACCTATGAACATGGCCATTGCTTTCTGTCA
661 -----+-----+-----+-----+-----+ 720
a M N T E K A R L L T Y E T W P L S F L S -

CCAGCAAAGCTGGCCAAAGCAGGCTTCTACTACATAGGACCTGGAGATAGAGTGGCCTGC
721 -----+-----+-----+-----+-----+ 780
a P A K L A K A G F Y Y I G P G D R V A C -

```

31/67

FIG. 5C

M-hiap-1

```

      TTTGCGTGGATGGGAACTGAGCAACTGGGAACGTAAGGATGATGCTATGTCAGAGCAC
781  - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 840
a    F A C D G K L S N W E R K D D A M S E H -

      CAGAGGCATTTCCTCCAGCTGTCCGTTCTTAAAGACTTGGGTCAGTCTGCTTCGAGATAC
841  - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 900
a    Q R H F P S C P F L K D L G Q S A S R Y -

      ACTGTCTCTAACCTGAGCATGCAGACACACAGCAGCCCGTATTAGAACATTTCTCTAACTGG
901  - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 960
a    T V S N L S M Q T H A A R I R T F S N W -

      CCTTCTAGTGCAC TAGTTCATCCAGGAAC TTGCAAGTGGGGCTTTATTATACAGGA
961  - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 1020
a    P S S A L V H S Q E L A S A G F Y Y T G -

      CACAGTGATGATGTC AAGTGT TATGCTGTGATGGTGGCTGAGGTGCTGGGAATCTGGA
1021 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 1080
a    H S D D V K C L C C D G G L R C W E S G -

      GATGACCCCTGGGTGGAACATGCCAAGTGGTTTCCAAGGTGTGAGTACTTGCTCAGAATC
1081 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 1140
a    D D P W V E H A K W F P R C E Y L L R I -

      AAAGGCCAAGAATTGTGAGCCCAAGTTCAAGCTGGCTATCCTCATCTACTTGAGCAGCTA
1141 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 1200
a    K G Q E F V S Q V Q A G Y P H L L E Q L -

```

FIG. 5D

M-hiap-1

TTATCTACGTACAGACTCCCGAAGATGAGAATGCAGACGCAGCAATCGTGCATTTTGGC
 1201 -----+-----+-----+-----+-----+ 1260
 L S T S D S P E D E N A D A A I V H F G -

 CCTGGAGAAAGTTCGGAAGATGTCGTCAATGATGACGACGCTGTGGTTAAAGCAGCCCTTG
 1261 -----+-----+-----+-----+-----+ 1320
 P G E S S E D V V M M S T P V V K A A L -

 GAAATGGGCTTCAGTAGGAGCCCTGGTGAGACAGACGGTTCAGTGGCAGATCCTGGCCACT
 1321 -----+-----+-----+-----+-----+ 1380
 E M G F S R S L V R Q T V Q W Q I L A T -

 GGTGAGAACTACAGGACCGTCAGTGACCTCGTTATAGGCTTACTCGATGCAGAGACGAG
 1381 -----+-----+-----+-----+-----+ 1440
 G E N Y R T V S D L V I G L L D A E D E -

 ATGAGAGAGGAGCAGATGGAGCAGGCGCGGAGGAGGAGTCTAGTATCTAGCCTA
 1441 -----+-----+-----+-----+-----+ 1500
 M R E E Q M E Q A A E E E E S D D L A L -

 ATCCGGAAGAACAAATGGTGCTTTTCCAAACATTGACGTGTGTGACACCAATGCTGTAT
 1501 -----+-----+-----+-----+-----+ 1560
 I R K N K M V L F Q H L T C V T P M L Y -

32/67

33/67

FIG. 5E

M-hiap-1

```

1561  TGCCTCCTAAGTCAAGGGCCATCACTGAACAGGAGTGCAATGCTGTGAAACAGAAACCA 1620
a  C L L S A R A I T E Q E C N A V K Q K P -
1621  CACACCTTACAAGCAAGCACACTGATTGATACTGTGTAGCAAAAGGAAACACTGCAGCA 1680
a  H T L Q A S T L I D T V L A K G N T A A -
1681  ACCTCATTCAGAAACTCCCTTCGGGAAATTGACCCCTGCGTTATACAGAGATATTTGTG 1740
a  T S F R N S L R E I D P A L Y R D I F V -
1741  CAACAGGACATTAGGAGTCTTCCACAGATGACATTGCAGCTCTACCAATGGAAGAACAG 1800
a  Q Q D I R S L P T D D I A A L P M E E Q -
1801  TTGCGGCCCTCCCGGAGGACAGAAATGTGTAAGTGTGTATGGACCGAGAGGTATCCATC 1860
a  L R P L P E D R M C K V C M D R E V S I -
1861  GTGTTTCATCCCTGTGGCCATCTGGTCGTGTGCAAGACTGCGCTCCCTCTCTGAGGAAG 1920
a  V F I P C G H L V V C K D C A P S L R K -

```

34/67

FIG. 5F

M-hiap-1

```

1921 TGTCCCATCTGTAGAGGAGGACCATCAAGGGCACAGTGGCACATTTCTCTCTGAAACAAGA 1980
a  - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +
   C P I C R G T I K G T V R T F L S * -
1981 CTAATGGTCCATGGCTGCAACTTCAGCCAGGAGGAAGTTCACCTGTCACTCCAGTTCCAT 2040
   - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +
   TCGGAACCTTGAGGCCAGCCTGGATAGCACGAGACACCGCCAAACACACAATAATAACAT
2041 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +
   GAAAAACTTTTGTCTGAAGTCAAGAATGAATGAATTACTTATATAATAATTTTAATTGGT
2101 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +
   TTCCCTTAAAGTGCTATTGTGTCCCAACTCAGAAAATGTTTCTGTAAACATATTTACA
2161 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +
   TACTACCTGCATCTAAAGTATTTCATATATTCATATATTCAGATGTCATGAGAGAGGGTTT
2221 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +
   TGTTCCTTGTTCCTGAAAAGCTGGTTTATCATCTGATCAGCATATACGCGCAACGGGCAG
2281 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +
   GGCTAGAATCCATGAACCAAGCTGCAAAGATCTCAGCTAAATAAGCGGAAAGATTGG
2341 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +
   AGAAACGAAAGGAAATTCTTTCCTGTCCCAATGTATACCTTCAGACTAATGACCTCTTCC
2401 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +
   TATCAAGCCTTCTA
2461 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - +

```

35/67

FIG. 6A

M-hiap-2

CTGTGTGGAGATCTATGTCCAGTGGTGAGAAACTTCATCTGGAAGTTTAAGCGGTCA
 1 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 60
 GAAATACTATTACTACTCATGACAAAACCTGTCTCCAGAGACTCGCCCAAGGTACCTTA
 61 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 120
 CACCCAAAACCTTAAACGTATAATGGAGAAGAGCACAATCTTGTCAAATTTGGACAAAGGA
 121 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 180
 M E K S T I L S N W T K E -
 b
 GAGCGAAGAAAAATGAAGTTTGACTTTTCGTGTGAACTCTACCGAATGTCTACATATTC
 181 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 240
 S E E K M K F D F S C E L Y R M S T Y S -
 b
 AGCTTTTCCAGGGAGTTCCTGTCTCAGAGAGGAGTCTGGCTCGTGGCTTTTATTA
 241 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 300
 A F P R G V P V S E R S L A R A G F Y Y -
 b
 TACAGGTGTGAATGACAAAGTCAAGTCTTCTGTGCGCCTGATGTTGGATAACTGAA
 301 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 360
 T G V N D K V K C F C C G L M L D N W K -
 b
 ACAAGGGACAGTCCTGTGTTGAAAAGCACAGACAGTTCTATCCCAGCTGCAGCTTTGTACA
 361 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 420
 Q G D S P V E K H R Q F Y P S C S F V Q -
 b

36/67

FIG. 6B

M-hiap-2

```

421  GACTCTGCTTTCAGCCAGTCTGCAGTCTCCATCTAAGAATATGTCTCTGTGAAAAGTAG
      T L L S A S L Q S P S K N M S P V K S R -
b

481  ATTTGCACATTGTCACCTCTGGAAACGAGGTGGCATTCACTCCAACCTGTGCTCTAGCCC
      F A H S S P L E R G G I H S N L C S S P -
b

541  TCTTAATTCTAGAGCAGTGGAAGACTTCTCATCAAGGATGCCCTGCAGCTATGCCAT
      L N S R A V E D F S S R M D P C S Y A M -
b

601  GAGTACAGAAGAGGCCAGATTCTTACTTACAGTATGTGGCCTTTAAGTTTCTGTCAACC
      S T E E A R F L T Y S M W P L S F L S P -
b

661  AGCAGAGCTGGCCAGAGCTGGCTTCTATTACATAGGCCCTGGAGACAGGGTGGCCTGTTT
      A E L A R A G F Y Y I G P G D R V A C F -
b

721  TGCCTGTGTGGGAACTGAGCAACTGGGAACCAAGGATTATGCTATGTCAGAGCACCG
      A C G G K L S N W E P K D Y A M S E H R -
b

```


37/67

FIG. 6C

M-hiap-2

```

CAGACATTTTCCCCACTGTCCATTCTGTGAAATACTTCAGAAACACAGAGTTTAGTAT
781  -----+-----+-----+-----+-----+-----+-----+ 840
      R H F P H C P F L E N T S E T Q R F S I -

ATCAAATCTAAGTATGCAGACACACTCTGCTCGATTGAGGACATTCTGTACTGCCACC
841  -----+-----+-----+-----+-----+-----+-----+ 900
      S N L S M Q T H S A R L R T F L Y W P P -

TAGTGTCCTGTTCAAGCCGAGCAGCTTGCAAGTCTGGATTCTATTACGTGGATCGCAA
901  -----+-----+-----+-----+-----+-----+-----+ 960
      S V P V Q P E Q L A S A G F Y Y V D R N -

TGATGATGTC AAGTGCCTTGTGTGATGGTGGCTTGAGATGTTGGGAACCTGGAGATGA
961  -----+-----+-----+-----+-----+-----+-----+ 1020
      D D V K C L C C D G G L R C W E P G D D -

CCCCTGGATAGAACACGCCAAATGGTTCCAAAGGTGTGAGTCTTGATACGGATGAAGGG
1021 -----+-----+-----+-----+-----+-----+-----+ 1080
      P W I E H A K W F P R C E F L I R M K G -

TCAGGAGTTTGTGTGATGAGATTCAAGCTAGATATCCTCATCTTCTTGAGCAGCTGTGTC
1081 -----+-----+-----+-----+-----+-----+-----+ 1140
      Q E F V D E I Q A R Y P H L L E Q L L S -

```

38/67

FIG. 6D

M-hiap-2

```

1141  CACTTCAGACACCCAGGAGAAGAAATGCTGACCCTACAGACAGAGTGCGTCATTTGG
      T S D T P G E E N A D P T E T V V H F G - 1200
b
1201  CCCTGGAGAAAGTTCGAAAGATGTCGTCAATGATGAGCAGCCTGTGGTTAAAGCAGCCCTT
      P G E S S K D V V M M S T P V V K A A L - 1260
b
1261  GGAAATGGGCTTCAGTAGGAGCCTGGTGAGACAGACGGTTCAGCGGAGATCCTGGCCAC
      E M G F S R S L V R Q T V Q R Q I L A T - 1320
b
1321  TGGTGAGAACTACAGGACCGTCAATGATATGTCTCAGTACTTTTGAATGCTGAAGATGA
      G E N Y R T V N D I V S V L L N A E D E - 1380
b
1381  GAGAAGAGAAGAGGAGAAGAAAGACAGACTGAAGAGATGGCATCAGGTGACTTATCACT
      R R E E E K E R Q T E E M A S G D L S L - 1440
b
1441  GATTCGGAAGAATAGATGGCCCTCTTTCAACAGTTGACACATGTCCTTCCTATCCTGGA
      I R K N R M A L F Q Q Q L T H V L P I L D - 1500
b

```

39/67

FIG. 6E

M-hiap-2

```

1501  TAATCTTCTTGAGCCAGTGTAAATTACAAAACAGGAACATGATATTATTAGACAGAAAAC
      N L L E A S V I T K Q E H D I I R Q K T -
b
1561  ACAGATACCCTTACAAGCAAGAGAGCTTATTGACACCCGTTTGTAGTCAAGGGAATGCTGC
      Q I P L Q A R E L I D T V L V K G N A A -
b
1621  AGCCAACATCTTCAAAAACCTCTCTGAAGGGAATTGACTCCACGTTATATGAAAACCTTATT
      A N I F K N S L K G I D S T L Y E N L F -
b
1681  TGTGAAAAGAATATGAAGTATATTCCAACAGAACGCTTTCAGGCTTGTCATTGGAAGA
      V E K N M K Y I P T E D V S G L S L E E -
b
1741  GCAGTGCAGAGATTACAAGAAGAACGAACTTGCAAAGTGTGTATGGACAGAGAGGTTTC
      Q L R R L Q E E R T C K V C M D R E V S -
b
1801  TATTGTGTTTCATCCGTTGGTCATCTAGTAGTCTGCCAGGAATGTGCCCTTCTCTAAG
      I V F I P C G H L V V C Q E C A P S L R -

```

40/67

FIG. 6F

M-hiap-2

GAAGTCCCCATCTGCAGGGGACAAATCAAGGGACTGTGCGCACATTCTCTCATGAGT 1920
b K C P I C R G T I K G T V R T F L S + -
GAAGATGGTCTGAAAGTATTGTTGGACATCAGAAGCTGTGAGAACAAAGAAATGAACCTAC 1980
TGATTTCAGCTCTTCAGCAGGACATTCTACTCTCTTCAAGATTAGTAATCTTGCTTTAT 1980
1981 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 2040
GAAGGTAGCATTGTATATTAAAGCTTAGTCTGTGCAAGGGAAGGTCATGCTGTTGAG 2040
2041 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 2100
CTACAGGACTGTGCTGTCCAGAGCAGGAGTTGGGATGCTTGCTGTATGTCCTTCAGGA 2100
2101 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 2160
CTTCTTGGGATTGGGAATTGGGGAAGCTTTGGAATCCAGTGATGTGGAGCTCAGAAA 2160
2161 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 2220
TCCTGGAACCCAGTGAATCTGGTACTCAGTAGATAGGGTACCCTGTACTTCTTGGTGCTTT 2220
2221 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 2280
TCCAGTCTGGGAAATAAGGAGGAATCTGCTGCTGGTAAATAATTGCTGGATGTGAGAAAT 2280
2281 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 2340
AGATGAAAGTGTTCGGGTGGGGCGTGATCAGTGTAGTGTGTCAGGGATGTATGCAG 2340
2341 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 2400
GCCAAACACTGTGTAG
2401 - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + - - - - - + 2416

41/67

FIG. 7A

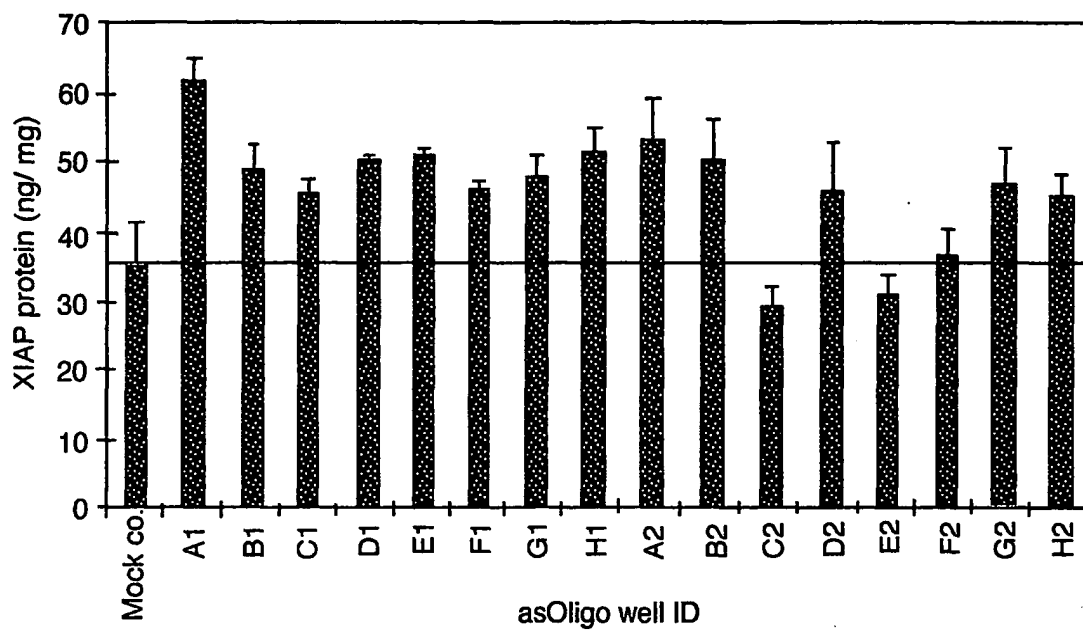
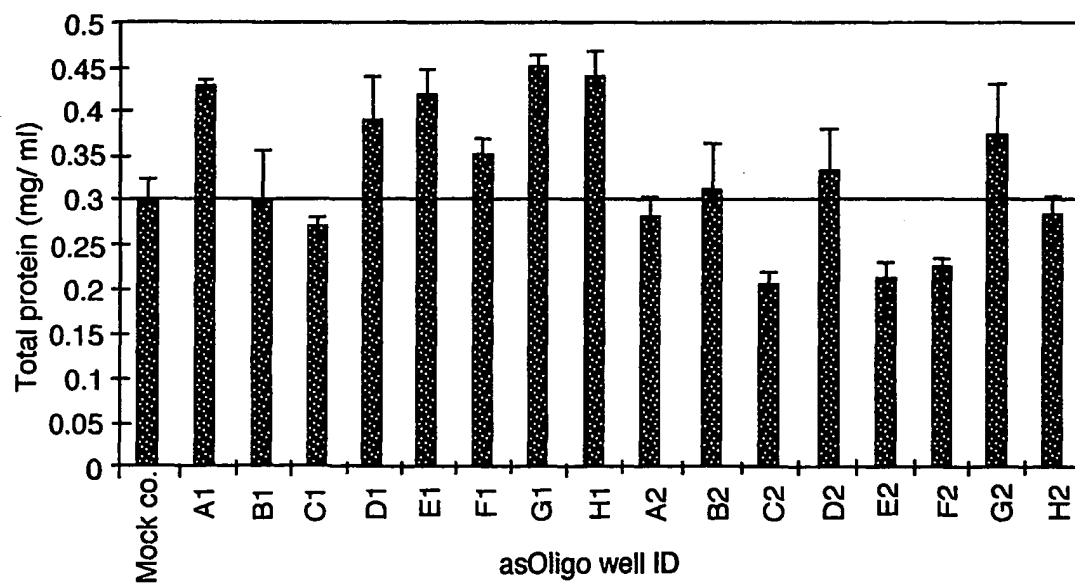


FIG. 7B



42/67

FIG. 7C

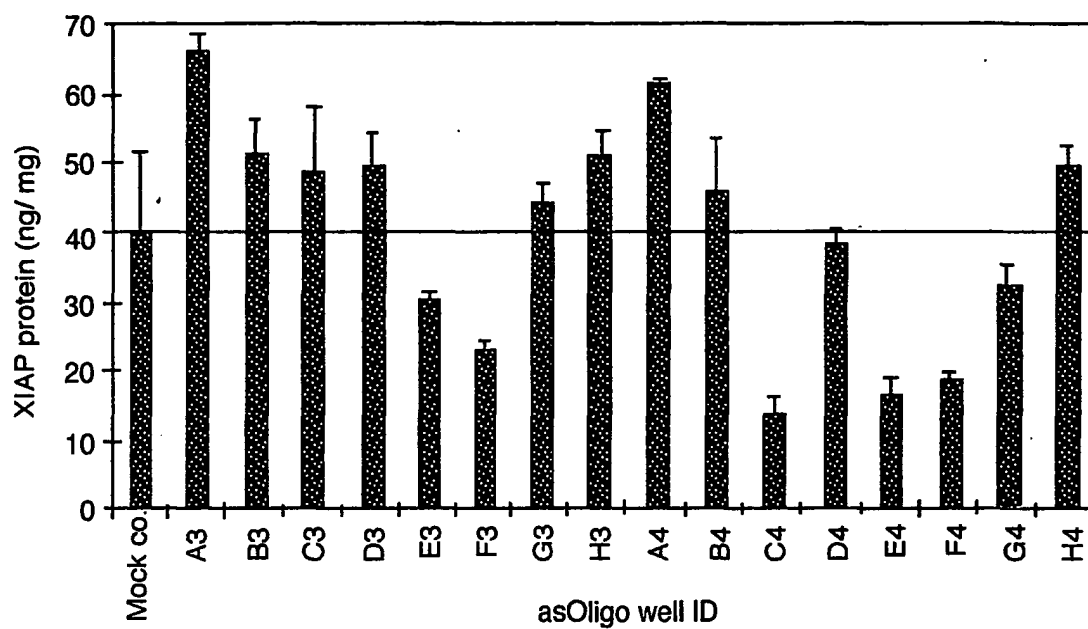
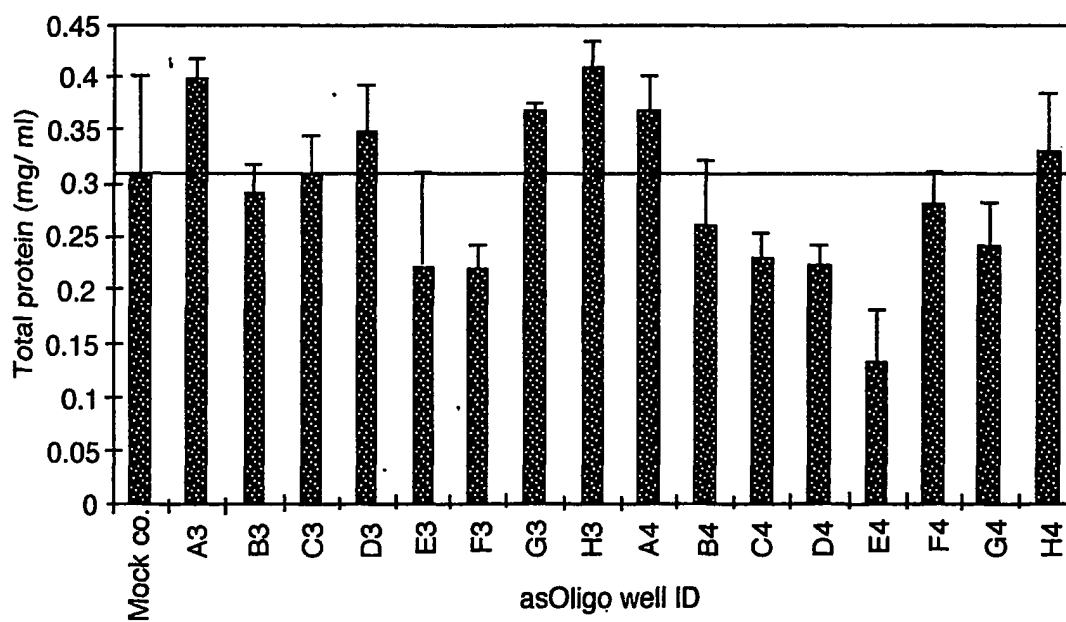


FIG. 7D



43/67

FIG. 7E

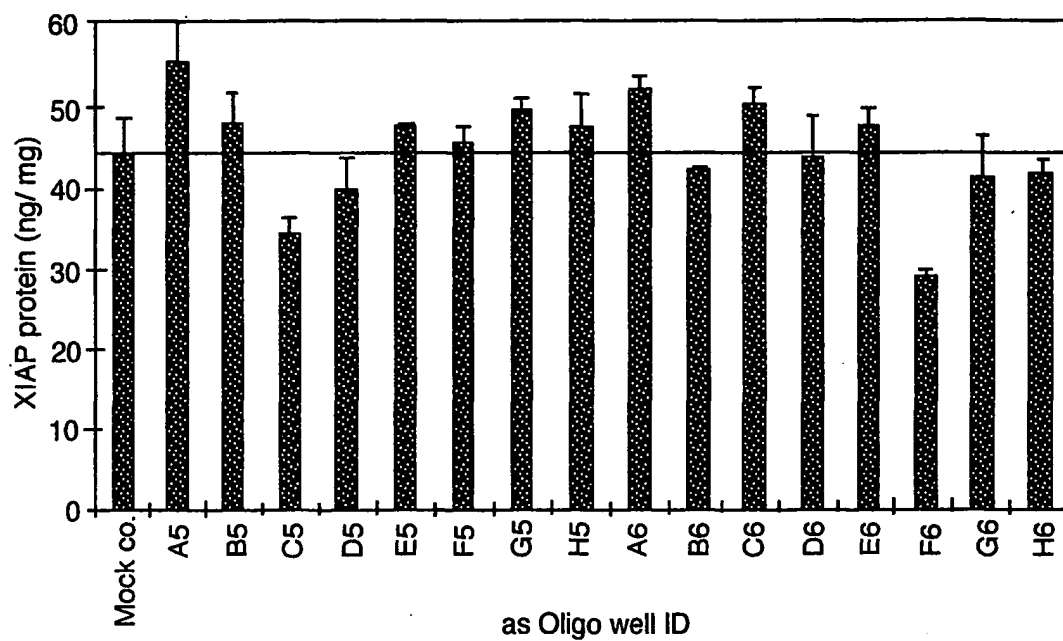
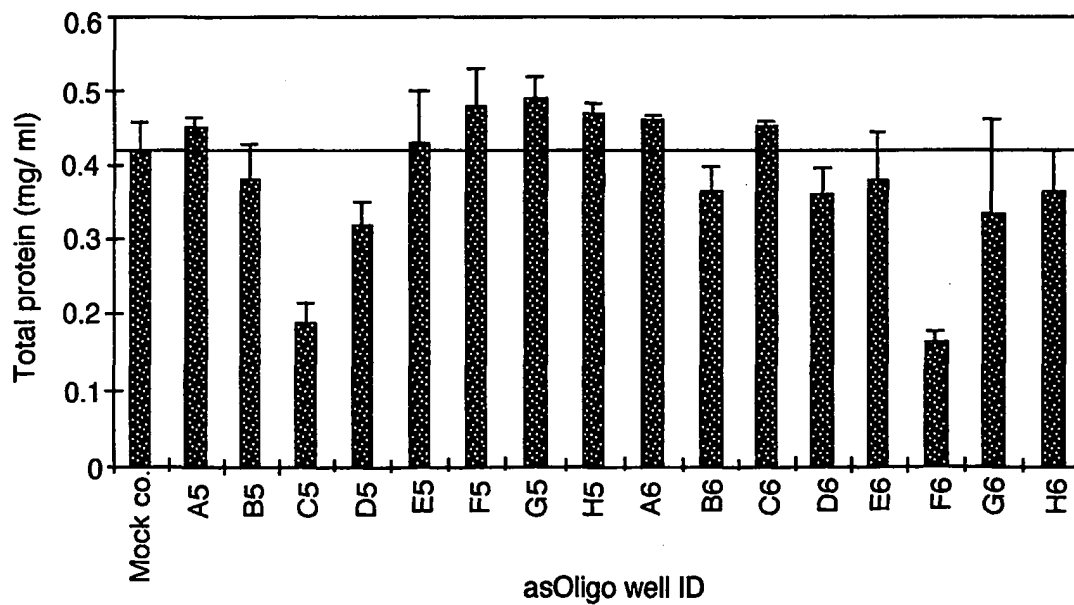


FIG. 7F



44/67

FIG. 7G

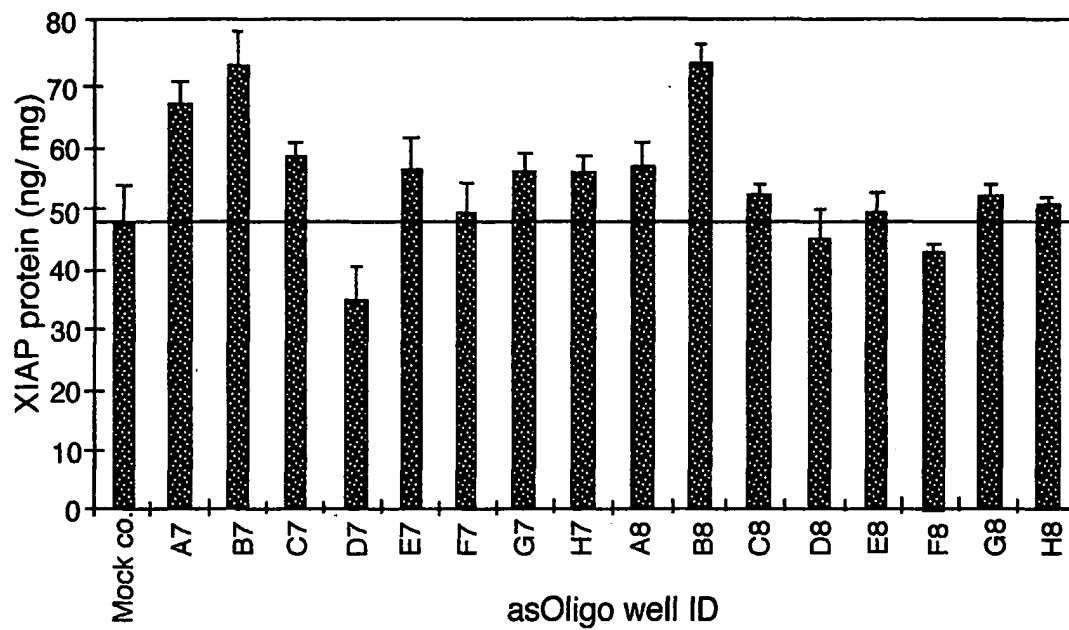
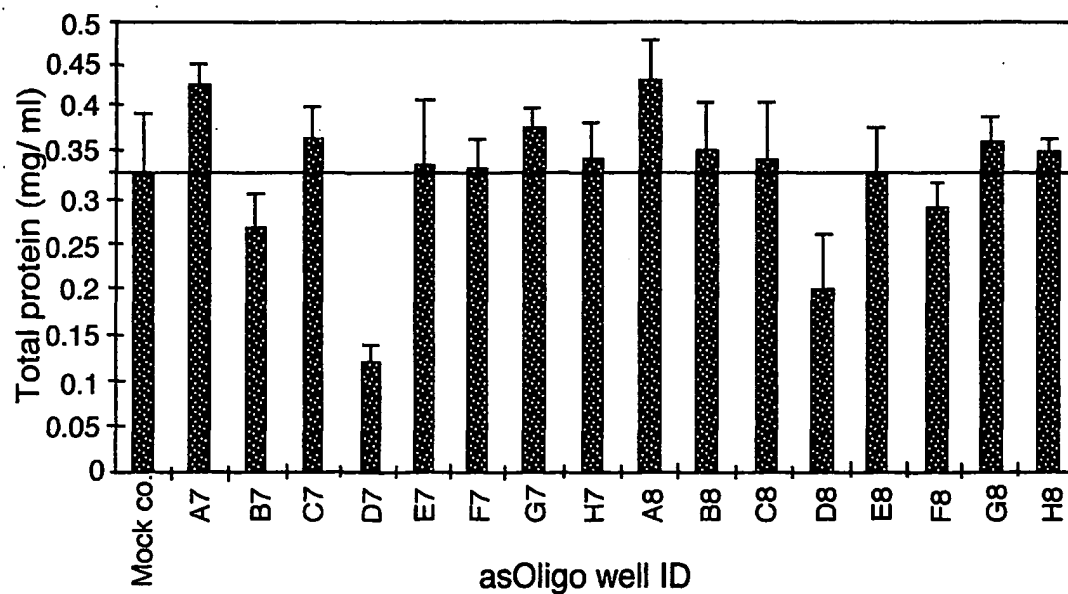


FIG. 7H



45/67

FIG. 7I

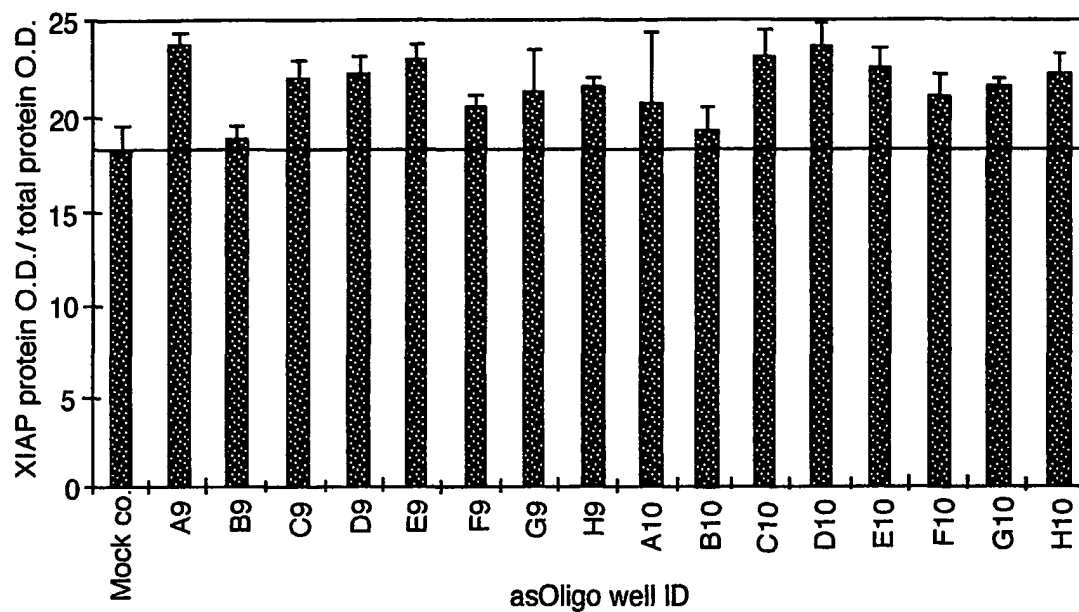
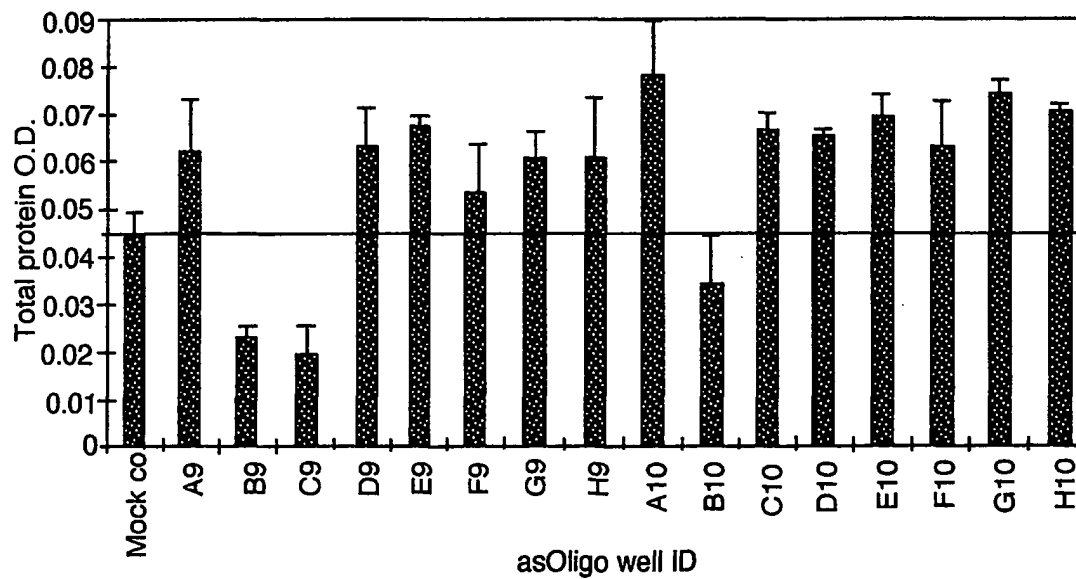


FIG. 7J



46/67

FIG. 7K

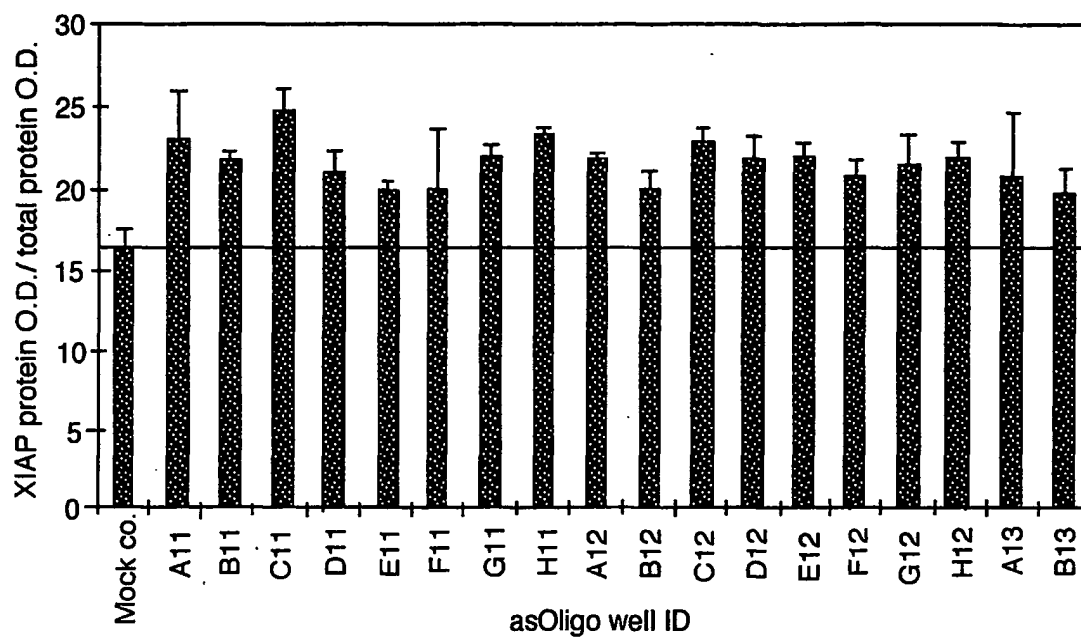
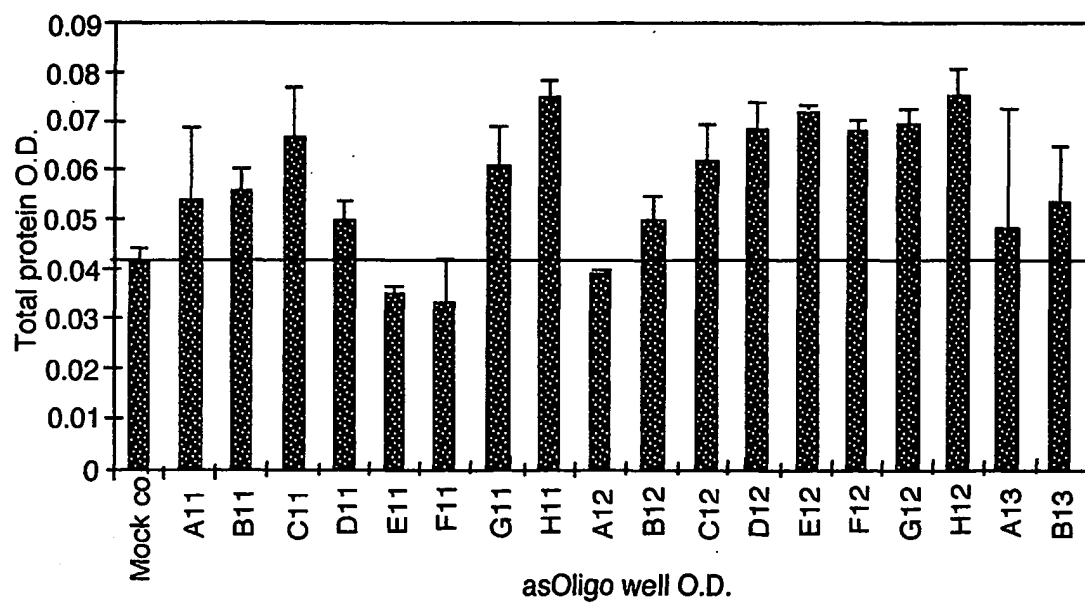


FIG. 7L



47/67

FIG. 8A

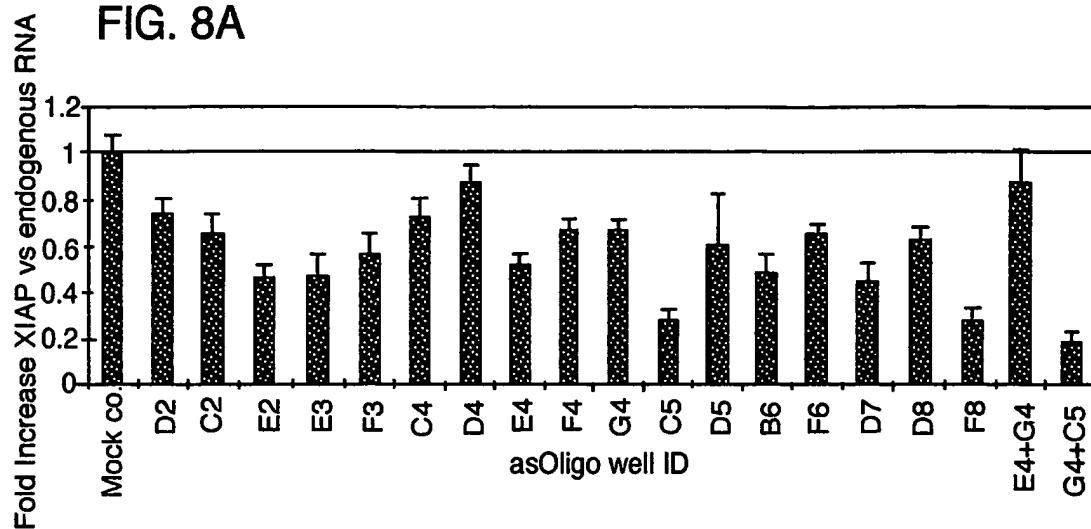


FIG. 8B

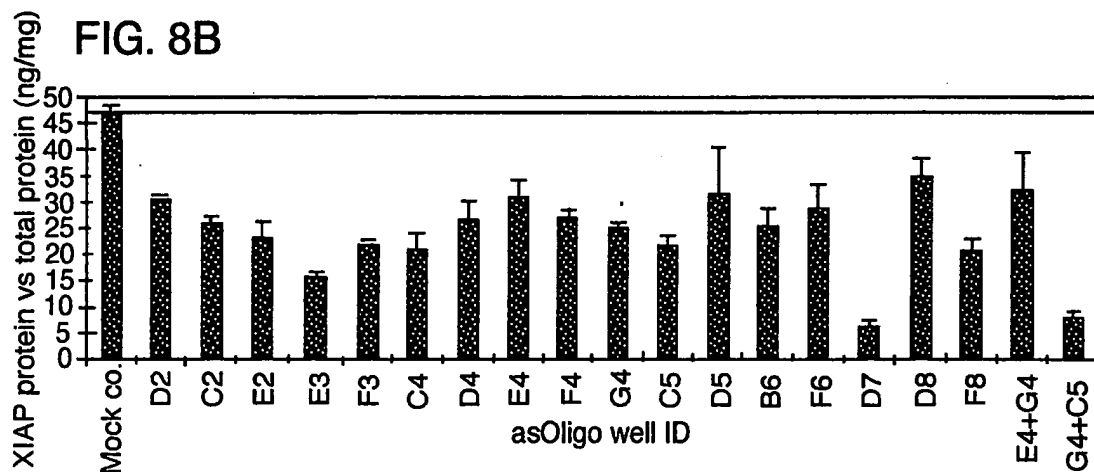
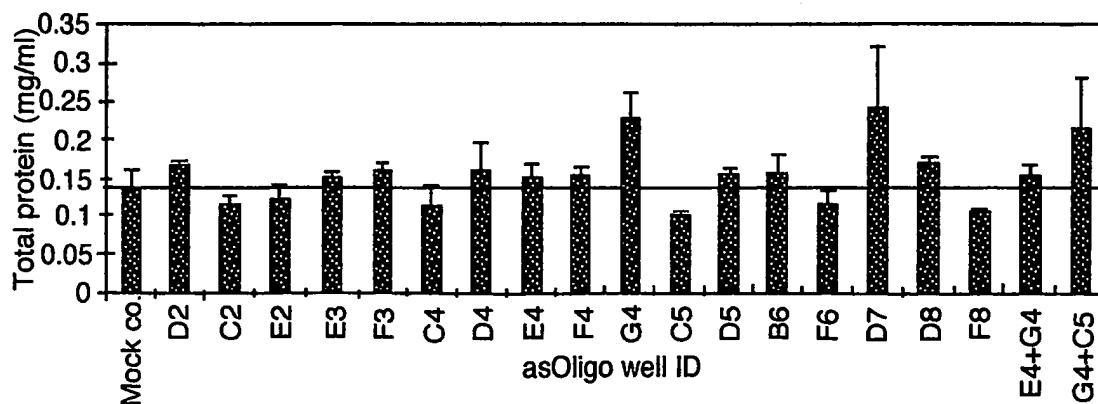
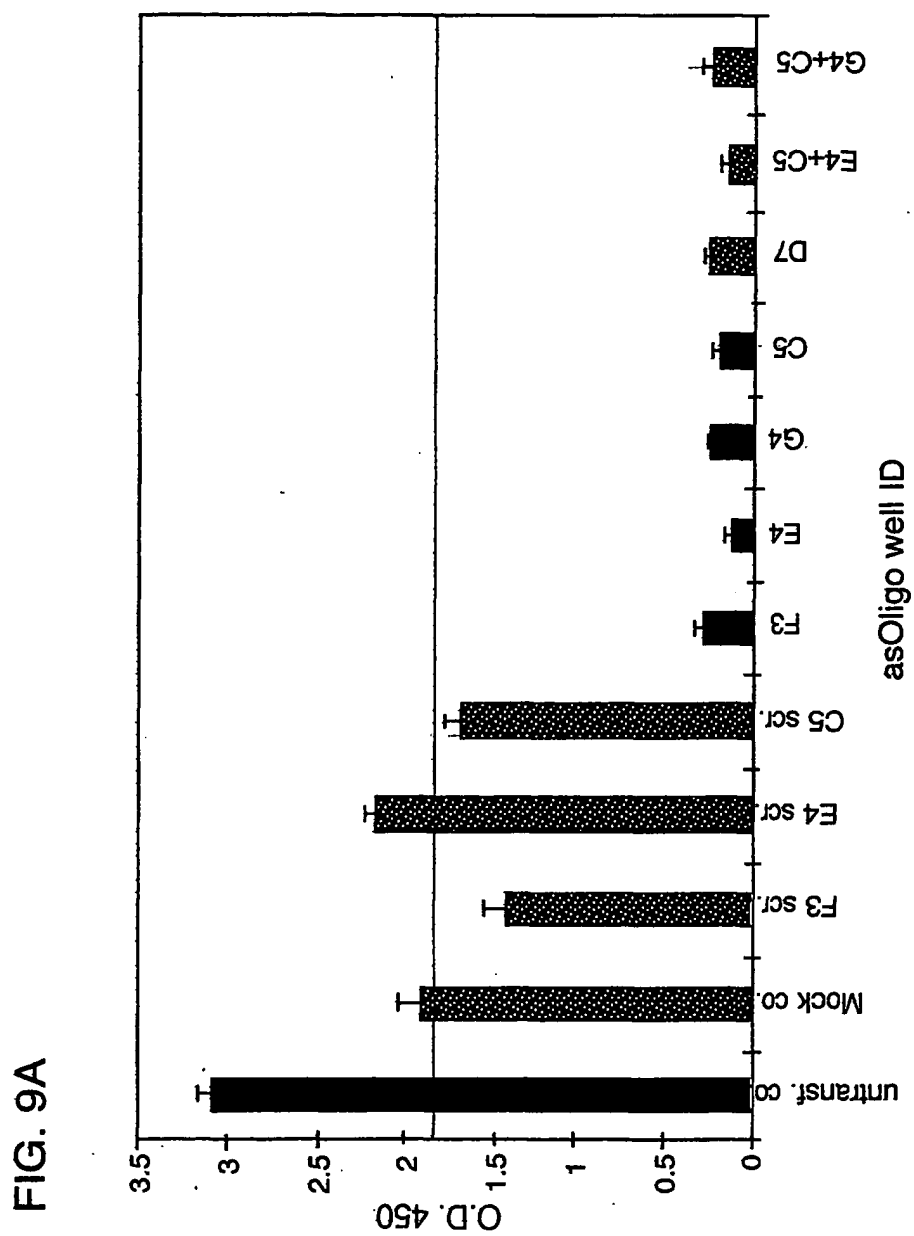


FIG. 8C



48/67



49/67

FIG. 9B

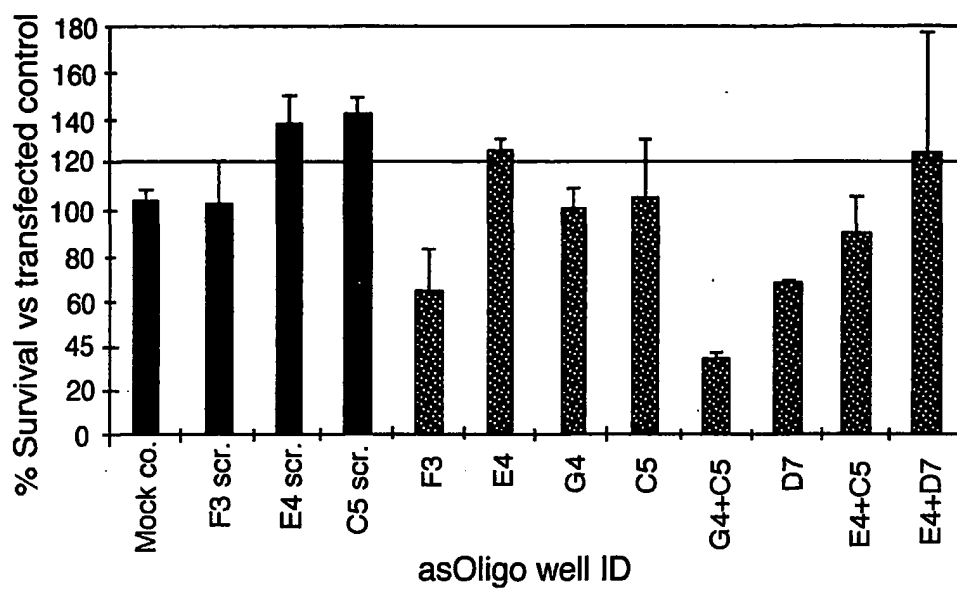
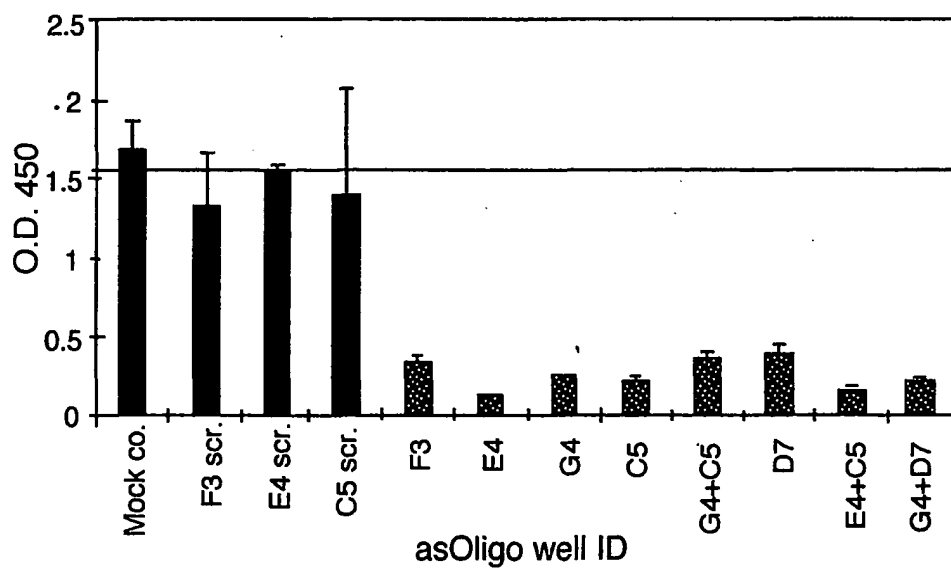
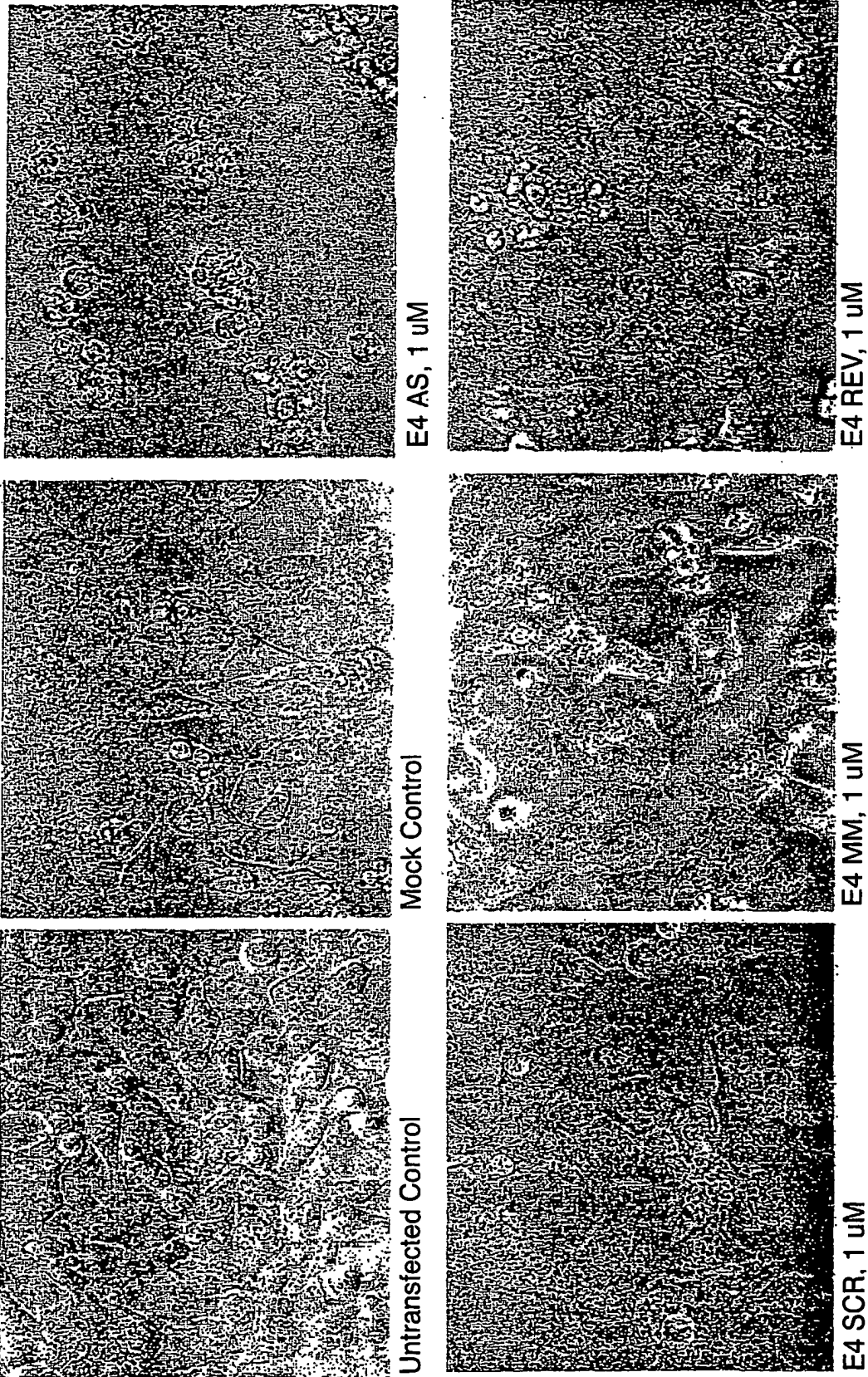


FIG. 9C

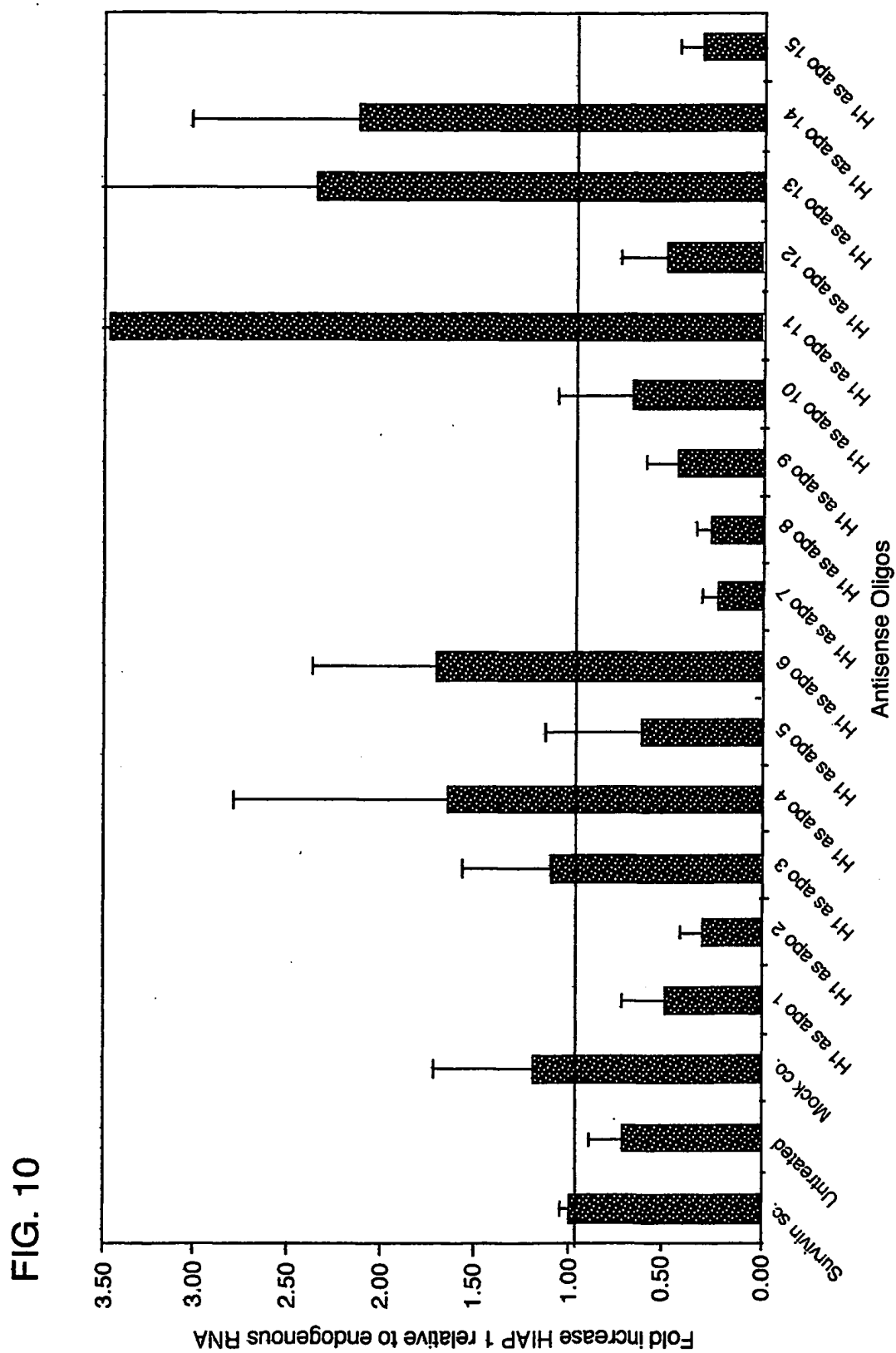


50/67

FIG. 9D



51/67



52/67

FIG. 11A

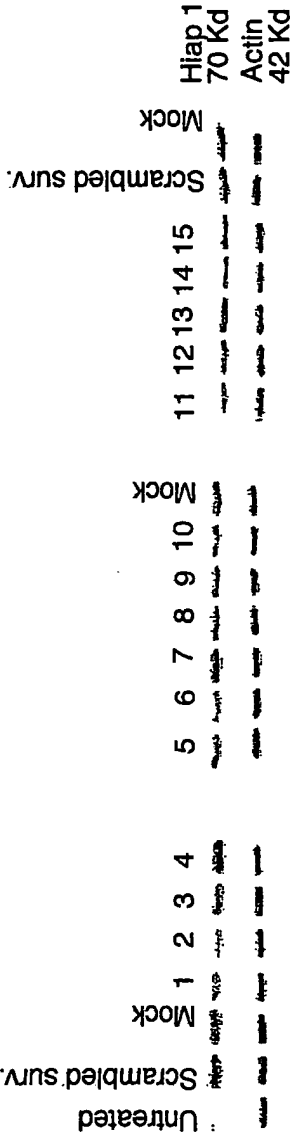
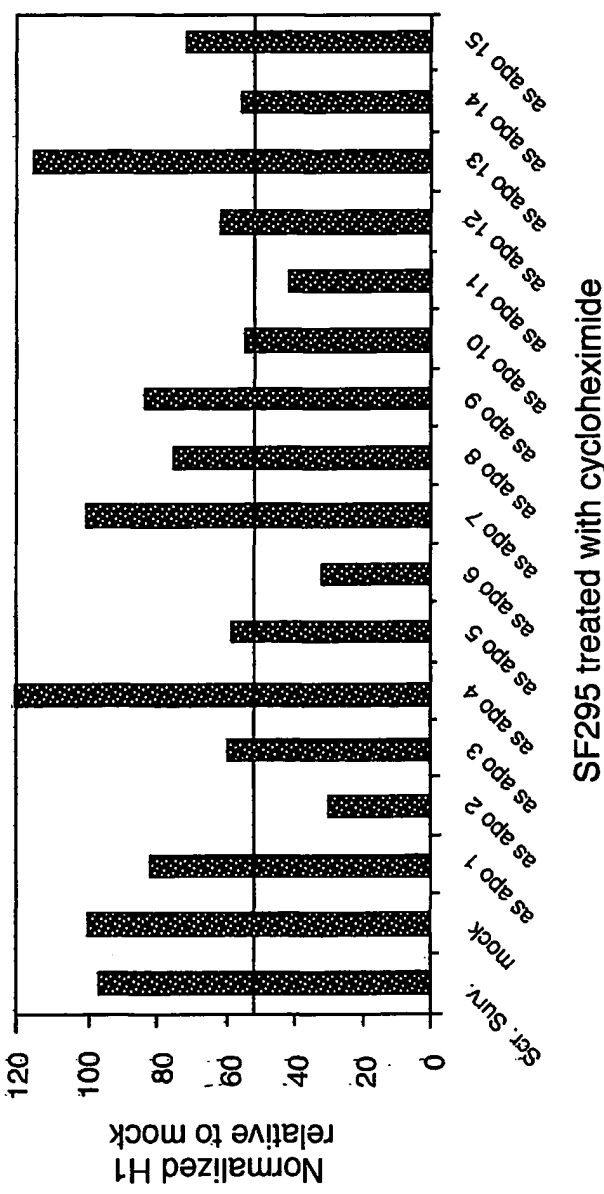
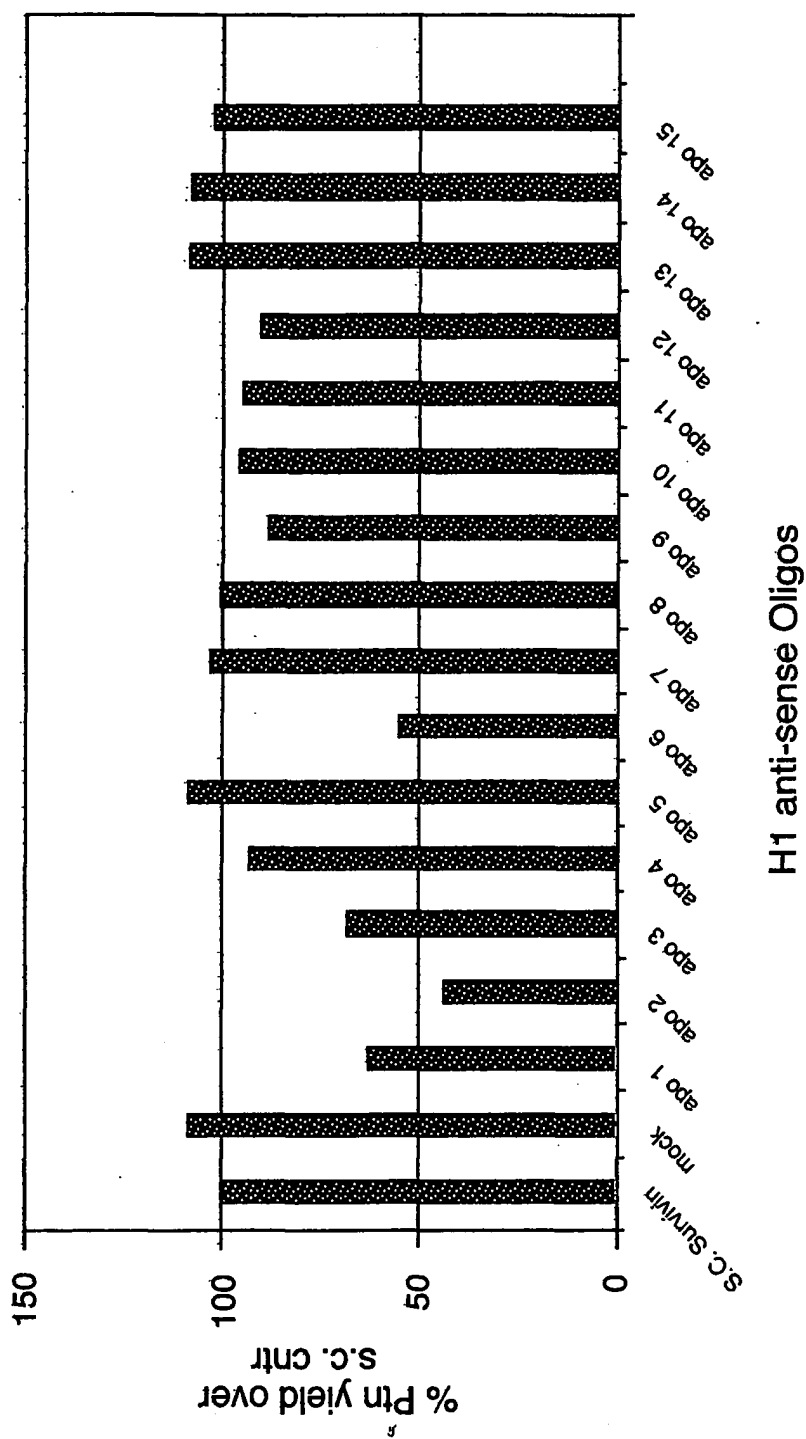


FIG. 11B



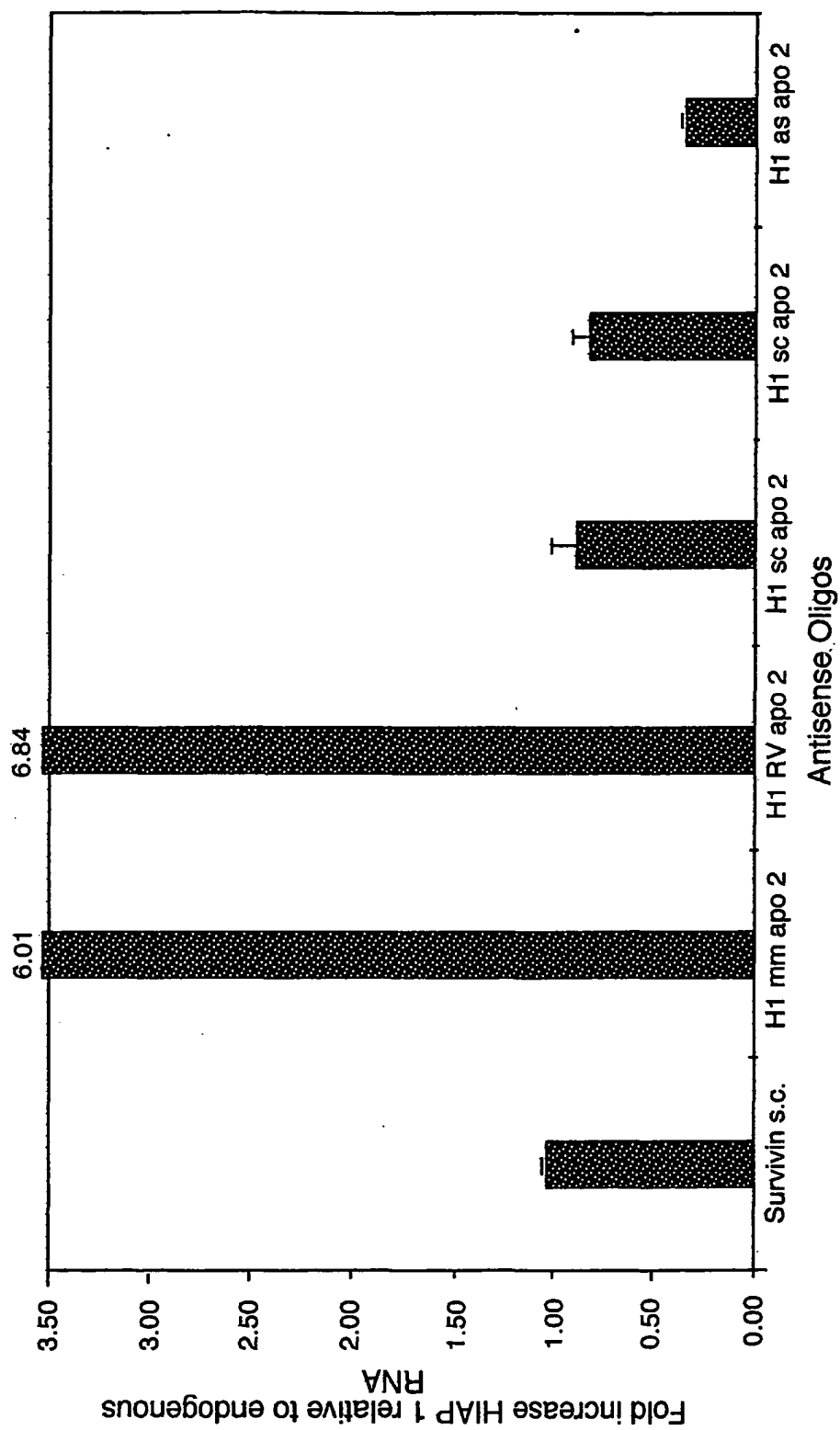
53/67

FIG. 12



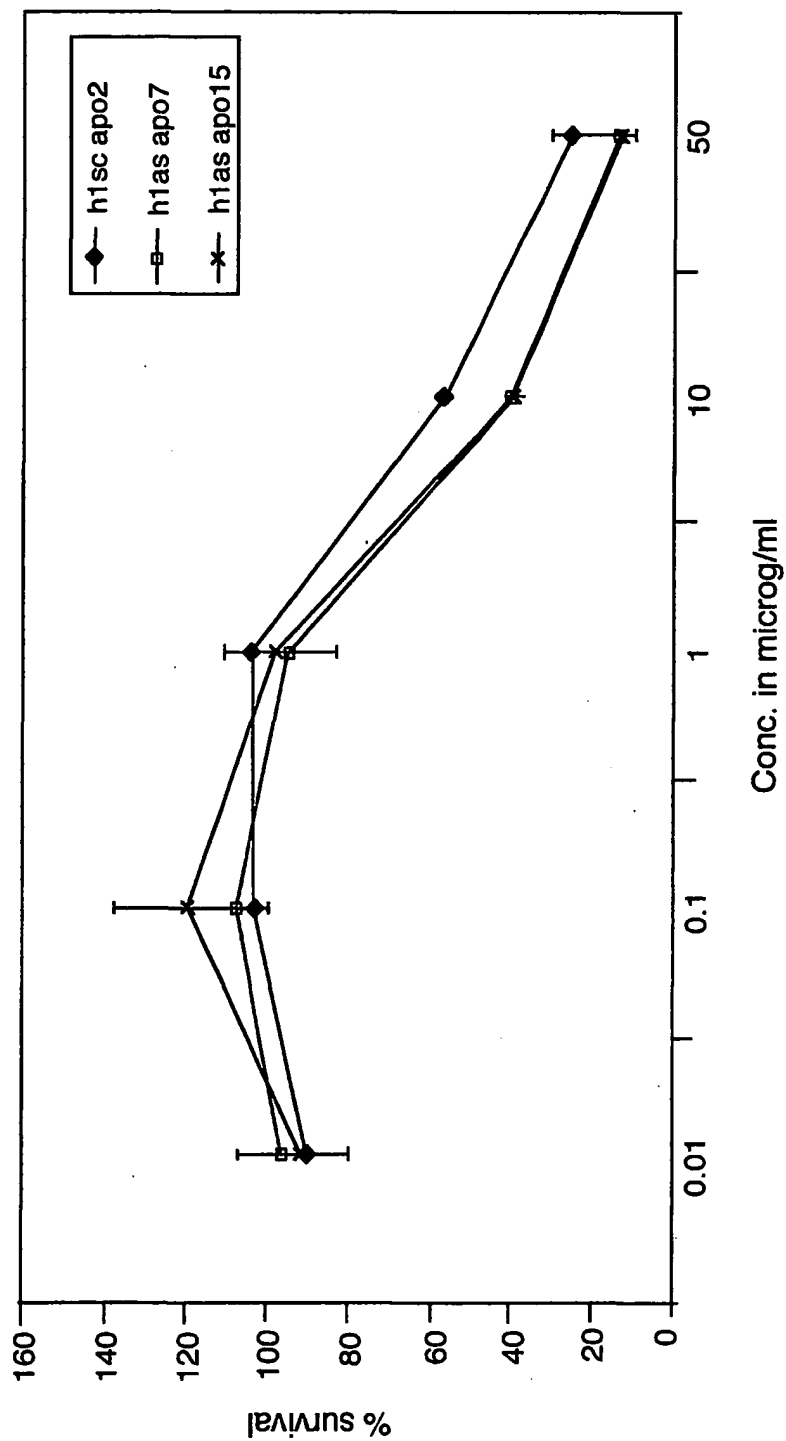
54/67

FIG. 13



55/67

FIG. 14



56/67

FIG. 15A

1 TTGCAGGTAC TTAGAATTTT TCCTGAGCCA CCCTCTAGAG GGCAGTGTTA
51 CATATATATC TGTAATTATC CAGTTACAAC AAAAAAAGGG CTCTCATTCA
101 TGCATGAAAA TCAGAAATAT TTCATACTCT TAAAGAACAC ATTGGAACCA
151 ATATTATGAT TAAACATAT TTTGCTAAGC AAAGAGATAT TAAAAATTAA
201 TTCATTAACA TTCTGAACAT TTTTAACTT GTAAAAACAA CTTTGATGCC
251 TTGAATATAT AATGATTCAT TATAACAATT ATGCATAGAT TTTAATAATC
301 TGCATATTTT ATGCTTTCAT GTTTTTCCTA ATTAATGATT TGACATGGTT
351 AATAATTATA ATATATTCTG CATCACAGTT TACATATTTA TGTAATAATA
401 GCATTTAAAA ATTATTAGTT TTATTCTGCC TGCTTAAATA TTACTTTCCT
451 CAAAAAGAGA AAACAAAAAT GCTAGATTTT ACTTTATGAC TTGAATGATG
501 TGGTAATGTC GAACTCTAGT ATTTAGAATT AGAATGTTTC TTAGCGGTGC
551 TGTAGTTATT TTTATGTCAT AAGTGGATAA TTTGTTAGCT CCTATAACAA
601 AAGTCTGTTG CTTGTGTTTC ACATTTTGGA TTTCTAATA TAATGTTCTC
651 TTTTAGAAA AGGTGGACAA GTCCTATTTT CAAGAGAAGA TGACTTTTAA
701 CAGTTTTGAA GGATCTAAAA CTTGTGTACC TGCAGACATC AATAAGGAAG
751 AAGAATTTGT AGAAGAGTTT AATAGATTAA AAACTTTGC TAATTTTCCA
801 AGTGGTAGTC CTGTTTCAGC ATCAACACTG GCACGAGCAG GGTTTCTTTA
851 TACTGGTGAA GGAGATACCG TCGGGTGCTT TAGTTGTCAT GCAGCTGTAG
901 ATAGATGGCA ATATGGAGAC TCAGCAGTTG GAAGACACAG GAAAGTATCC
951 CCAAATTGCA GATTTATCAA CGGCTTTTAT CTTGAAAATA GTGCCACGCA

57/67

FIG. 15B

1001 GTCTACAAAT TCTGGTATCC AGAATGGTCA GTACAAAGTT GAAAACATATC
1051 TGGGAAGCAG AGATCATTTT GCCTTAGACA GGCCATCTGA GACACATGCA
1101 GACTATCTTT TGAGAACTGG GCAGGTTGTA GATATATCAG ACACCATATA
1151 CCCGAGGAAC CCTGCCATGT ATTGTGAAGA AGCTAGATTA AAGTCCTTTC
1201 AGAACTGGCC AGACTATGCT CACCTAACCC CAAGAGAGTT AGCAAGTGCT
1251 GGACTCTACT ACACAGGTAT TGGTGACCAA GTGCAGTGCT TTTGTTGTGG
1301 TGGAAAAC TG AAAAATTGGG AACCTTGTGA TCGTGCCTGG TCAGAACACA
1351 GGCGACACTT TCCTAATTGC TTCTTTGTTT TGGGCCGGAA TCTTAATATT
1401 CGAAGTGAAT CTGATGCTGT GAGTTCTGAT AGGAATTTCC CAAATTCAAC
1451 AAATCTTCCA AGAAATCCAT CCATGGCAGA TTATGAAGCA CGGATCTTTA
1501 CTTTGGGAC ATGGATATAC TCAGTTAACA AGGAGCAGCT TGCAAGAGCT
1551 GGATTTTATG CTTTAGGTGA AGGTGATAAA GTAAAGTGCT TTCACTGTGG
1601 AGGAGGGCTA ACTGATTGGA AGCCCAGTGA AGACCCTTGG
GAACAACATG
1651 CTAAATGGTA TCCAGGGTGC AAATATCTGT TAGAACAGAA
GGGACAAGAA
1701 TATATAAACA ATATTCATTT AACTCATTCA CTTGAGGAGT GTCTGGTAAG
1751 AACTACTGAG AAAACACCAT CACTAACTAG AAGAATTGAT GATACCATCT
1801 TCCAAAATCC TATGGTACAA GAAGCTATAC GAATGGGGTT CAGTTTCAAG
1851 GACATTAAGA AAATAATGGA GGAAAAAATT CAGATATCTG
GGAGCAACTA
1901 TAAATCACTT GAGGTTCTGG TTGCAGATCT AGTGAATGCT CAGAAAGACA
1951 GTATGCAAGA TGAGTCAAGT CAGACTTCAT TACAGAAAGA GATTAGTACT
2001 GAAGAGCAGC TAAGGCGCCT GCAAGAGGAG AAGCTTTGCA
AAATCTGTAT

58/67

FIG. 15C

2051 GGATAGAAAT ATTGCTATCG TTTTGTTC TTGTGGACAT CTAGTCACTT
2101 GTAAACAATG TGCTGAAGCA GTTGACAAGT GTCCCATGTG CTACACAGTC
2151 ATTACTTTCA AGCAAAAAAT TTTTATGTCT TAATCTAACT CTATAGTAGG
2201 CATGTTATGT TGTTCTTATT ACCCTGATTG AATGTGTGAT GTGAACTGAC
2251 TTTAAGTAAT CAGGATTGAA TTCCATTAGC ATTTGCTACC AAGTAGGAAA
2301 AAAAATGTAC ATGGCAGTGT TTTAGTTGGC AATATAATCT TTGAATTTCT
2351 TGATTTTTCA GGGTATTAGC TGTATTATCC ATTTTTTTTA CTGTTATTTA
2401 ATTGAAACCA TAGACTAAGA ATAAGAAGCA TCATACTATA ACTGAACACA
2451 ATGTGTATTC ATAGTATACT GATTTAATTT CTAAGTGTA GTGAATTAAT
2501 CATCTGGATT TTTTATTCTT TTCAGATAGG CTTAACAAAT GGAGCTTTCT
2551 GTATATAAAT GTGGAGATTA GAGTTAATCT CCCCAATCAC ATAATTTGTT
2601 TTGTGTGAAA AAGGAATAAA TTGTTCCATG CTGGTGGAAA GATAGAGATT
2651 GTTTTTAGAG GTTGGTTGTT GTGTTTTAGG ATTCTGTCCA TTTTCTTTA
2701 AAGTTATAAA CACGTACTTG TGCGAATTAT TTTTTTAAAG TGATTTGCCA
2751 TTTTGAAAG CGTATTTAAT GATAGAATAC TATCGAGCCA ACATGTACTG
2801 ACATGGAAAG ATGTCAAAGA TATGTTAAGT GTAAAATGCA
AGTGGCAAAA
2851 CACTATGTAT AGTCTGAGCC AGATCAAAGT ATGTATGTTT TTAATATGCA
2901 TAGAACAAAA GATTTGGAAA GATATACACC AAAGTGTTAA ATGTGGTTTC
2951 TCTTCGGGGA GGGGGGGATT GGGGGAGGGG CCCCATAGGG GTTTTATAGG

59/67

FIG. 16A

1 TTGCTCTGTC ACCCAGTTTG GAGTGCAGTT ATGCAGTCTC
ACACTGCAAG

51 CTCTGCCTCA TGGGCTCAAG TGAACCTCCT GCCTCAGCCT
CTCAAGTAGC

101 TGGGACCACA GGCAGGTGCC ACCATGTCTG GCTAATTTT
GAGTTTCTTT

151 GTAGAGATGG TGTTTTGCCA AGTCACCCAG TTTGAGGCTG
GTCTCAAACA

201 CCTGGGCTCA AGCAATCCAT CTACCTCAGC CTCCCAAAGT
GCTGGGATTA

251 CAGGAGTGAG CCATGGCATG AGGCCTTG TG GGGTGTCTCT
TTTAAATGAA

301 AGCATACTCT GTTTACGTAT TTGATATGAA GGAATATCCT
TCCTTTCCAC

351 AAAGACAAAA ATTATCCTAT TTTTCTCAAA ACATATGTCC
TTTTTCTCTA

401 CTTTTCATTT TTGTTACTTT TGATGGACAC ATGTGTTACA
TTGATTTCAC

451 TTTCTCATAA TTCTGCTGTA AGAAAAACAA TAGTGCCAGT
TCAATGACAA

501 ATAGCAACAG TCTGTTATTG CTAGACTGTT ACTGTTAGTG
GAGACTACCA

551 GAACAGTCAG TCCCAGTGTC AGGGAATCAA AGAGAACATG
TTCCCTCTCT

601 AAAGGGCACA GCTGCTGCTC AGCTTTAGCT GATTGCTGCC
CTGCAGGACT

651 ATAGGCCCCAG TGTTGCTAGA TCTTTTGATG TTTCAAGAGA
AGCTTGGAAT

701 CTAGAATGTG ATGGGAAGTC TCTTACATTT AAACATGTTG
GCAATTAATG

60/67

FIG. 16B

751 GTAAGATTTA AAAATACTGT GGTCCAAGAA AAAAATGGAT
TTGGAAACTG

801 GATTAAATTC AAATGAGGCA TGCAGATTAA TCTACAGCAT
GGTACAATGT

851 GAATTTTCTG GTTCTTTAA TTGCACTGTA ATTAGGTAAG
ATGTTAGCTT

901 TGGGGAAGCT AAGTGCAGAG TATGCAGAAA CTATTATTTT
TGTAAGTTTT

951 CTCTAAGTAT AAATAAATTT CAAAATAAAA ATAAAAACTT
AGTAAAGAAC

1001 TATAATGCAA TTCTATGTAA GCCAAACATA ATATGTCTTC
CAGTTTGAAA

1051 CCTCTGGGTT TTATTTTATT TTATTTTATT TTTGAGACAG
AGTCTTGCTG

1101 TGTCACCCAG GCTGGAGTGT AGTGGCACTA TTTCGGCCCA
CTGCAACCTC

1151 CACCTCCCAG GCTCAAATGA TTCTCCTGCC TCAGCCTCCG
GAGTAGCTGG

1201 GATTACAGGC GCGTACCACC ACACCCAGCT AATTTTGTGTA
TTTTTAGTAG

1251 AGATGGGGTT TCACCATTTT GGCCAGGCTG GTTTTGAAC
CTGACCTCA

1301 AGTGATCCAC TTGTCTTGGC CTCCCAAAT GCTGGGATTA
CAGGCGTGAG

1351 CCACTGCACC AGGCAGAGGC CTCTGTTTTT TATCTCTTTT
TGGCCTCTAC

1401 AGTGCCTAGT AAAGCACCTG ATACATGGTA AACGATCAGT
AATTACTAGT

1451 ACTCTATTTT GGAGAAAATG ATTTTTTAAA AAGTCATTGT
GTTCCATCCA

FIG. 16C 61/67

1501 TGAGTCGTTT GAGTTTTAAA ACTGTCTTTT TGTTTGTTT
TGAACAGGTT

1551 TACAAAGGAG GAAAACGACT TCTTCTAGAT TTTTTTTCA
GTTTCTTCTA

1601 TAAATCAAAA CATCTCAAAA TGGAGACCTA AAATCCTTAA
AGGGACTTAG

1651 TCTAATCTCG GGAGGTAGTT TTGTGCATGG GTAAACAAAT
TAAGTATTAA

1701 CTGGTGTTTT ACTATCCAAA GAATGCTAAT TTTATAAACA
TGATCGAGTT

1751 ATATAAGGTA TACCATAATG AGTTTGATTT TGAATTTGAT
TTGTGGAAAT

1801 AAAGGAAAAG TGATTCTAGC TGGGGCATAT TGTTAAAGCA
TTTTTTTCAG

1851 AGTTGGCCAG GCAGTCTCCT ACTGGCACAT TCTCCATTA
TGTAGAATAG

1901 AAATAGTACC TGTGTTTGGG AAAGATTTTA AAATGAGTGA
CAGTTATTG

1951 GAACAAAGAG CTAATAATCA ATCCACTGCA AATTAAAGAA
ACATGCAGAT

2001 GAAAGTTTTG ACACATTAAA ATACTTCTAC AGTGACAAAG
AAAAATCAAG

2051 AACAAAGCTT TTTGATATGT GCAACAAATT TAGAGGAAGT
AAAAAGATAA

2101 ATGTGATGAT TGGTCAAGAA ATTATCCAGT TATTTACAAG
GCCACTGATA

2151 TTTTAAACGT CCAAAAGTTT GTTTAAATGG GCTGTTACCG
CTGAGAATGA

2201 TGAGGATGAG AATGATGGTT GAAGGTTACA TTTTAGGAAA
TGAAGAAACT

2251 TAGAAAATTA ATATAAAGAC AGTGATGAAT ACAAAGAAGA

FIG. 16D

62/67

TTTTTATAAC

2301 AATGTGTAAA ATTTTGGCC AGGGAAAGGA ATATTGAAGT
TAGATACAAT

2351 TACTTACCTT TGAGGGAAAT AATTGTTGGT AATGAGATGT
GATGTTTCTC

2401 CTGCCACCTG GAAACAAAGC ATTGAAGTCT GCAGTTGAAA
AGCCCAACGT

2451 CTGTGAGATC CAGGAAACCA TGCTTGCAA CCACTGGTAA
AAAAAAAAAA

2501 AAAAAAAAAA AAAGCCACAG TGACTTGCTT ATTGGTCATT
GCTAGTATTA

2551 TCGACTCAGA ACCTCTTTAC TAATGGCTAG TAAATCATAA
TTGAGAAATT

2601 CTGAATTTTG ACAAGGTCTC TGCTGTTGAA ATGGTAAATT
TATTATTTT

2651 TTTGTCATGA TAAATTCTGG TTCAAGGTAT GCTATCCATG
AAATAATTTC

2701 TGACCAAAAC TAAATTGATG CAATTTGATT ATCCATCTTA
GCCTACAGAT

2751 GGCATCTGGT AACTTTTGAC TGTTTTAAAA AATAAATCCA
CTATCAGAGT

2801 AGATTTGATG TTGGCTTCAG AAACATTTAG AAAACAAAA
GTTCAAAAAT

2851 GTTTTCAGGA GGTGATAAGT TGAATAACTC TACAATGTTA
GTTCTTTGAG

2901 GGGGACAAAA AATTTAAAAT CTTTGAAAGG TCTTATTTTA
CAGCCATATC

2951 TAAATTATCT TAAGAAAATT TTAAACAAAG GGAATGAAAT
ATATATCATG

3001 ATTCTGTTTT TCCAAAAGTA ACCTGAATAT AGCAATGAAG
TTCAGTTTTG

FIG. 16E

63/67

3051 TTATTGGTAG TTTGGGCAGA GTCTCTTTT GCAGCACCTG
TTGTCTACCA

3101 TAATTACAGA GGACATTTC ATGTTCTAGC CAAGTATACT
ATTAGAATAA

3151 AAAA ACTTAA CATTGAGTTG CTTCAACAGC ATGAAACTGA
GTCCAAAAGA

3201 CCAAATGAAC AAACACATTA ATCTCTGATT ATTTATTTTA
AATAGAATAT

3251 TTAATTGTGT AAGATCTAAT AGTATCATTA TACTTAAGCA
ATCATATTCC

3301 TGATGATCTA TGGGAAATAA CTATTATTTA ATTAATATTG
AAACCAGGTT

3351 TTAAGATGTG TTAGCCAGTC CTGTTACTAG TAAATCTCTT
TATTTGGAGA

3401 GAAATTTTAG ATTGTTTTGT TCTCCTTATT AGAAGGATTG
TAGAAAGAAA

3451 AAAATGACTA ATTGGAGAAA AATTGGGGAT ATATCATATT
TCACTGAATT

3501 CAAAATGTCT TCAGTTGTAA ATCTTACCAT TATTTTACGT
ACCTCTAAGA

3551 AATAAAAGTG CTTCTAATTA AAATATGATG TCATTAATTA
TGAAATACTT

3601 CTTGATAACA GAAGTTTTAA AATAGCCATC TTAGAATCAG
TGAAATATGG

3651 TAATGTATTA TTTTCCTCCT TTGAGTNAGG TCTTGTGCTT
TTNTTCCTG

3701 GCCACTAAAT NTCACCATNT CCAANAAGCA AANTAAACCT
ATTCTGAATA

3751 TTTTGCTGT GAAACACTTG NCAGCAGAGC TTTCCCNCCA
TGNNAGAAGC

FIG. 16F

64/67

3801 TTCATGAGTC ACACATTACA TCTTTGGGTT GATTGAATGC
CACTGAAACA

3851 TTTCTAGTAG CCTGGAGNAG TTGACCTACC TGTGGAGATG
CCTGCCATTA

3901 AATGGCATCC TGATGGCTTA ATACACATCA CTCTTCTGTG
NAGGGTTTTA

3951 ATTTTCAACA CAGCTTACTC TGTAGCATCA TGTTTACATT
GTATGTATAA

4001 AGATTATACN AAGGTGCAAT TGTGTATTTC TTCCTTAAAA
TGTATCAGTA

4051 TAGGATTTAG AATCTCCATG TTGAAACTCT AAATGCATAG
AAATAAAAAT

4101 AATAAAAAAT TTTTCATTTT GGCTTTTCAG CCTAGTATTA
AAACTGATAA

4151 AAGCAAAGCC ATGCACAAAA CTACCTCCCT AGAGAAAGGC
TAGTCCCTTT

4201 TCTTCCCCAT TCATTTTATT ATGAACATAG TAGAAAACAG
CATATTCTTA

4251 TCAAATTTGA TGAAAAGCGC CAACACGTTT GAACTGAAAT
ACGACTTGTC

4301 ATGTGAACTG TACCGAATGT CTACGTATTC CACTTTTCCT
GCTGGGGTTC

4351 CTGTCTCAGA AAGGAGTCTT GCTCGTGCTG GTTTCTATTA
CACTGGTGTG

4401 AATGACAAGG TCAAATGCTT CTGTTGTGGC CTGATGCTGG
ATAACTGGAA

4451 AAGAGGAGAC AGTCCTACTG AAAAGCATAA AAAGTTGTAT
CCTAGCTGCA

4501 GATTCGTTCA GAGTCTAAAT TCCGTTAACA ACTTGGAAGC
TACCTCTCAG

4551 CCTACTTTTC CTTCTTCAGT AACACATTCC ACACACTCAT

65/67

FIG. 16G

TACTTCCGGG

4601 TACAGAAAAC AGTGGATATT TCCGTGGCTC TTATTCAAAC
TCTCCATCAA4651 ATCCTGTAAA CTCCAGAGCA AATCAAGAAT TTTCTGCCTT
GATGAGAAGT4701 TCCTACCCCT GTCCAATGAA TAACGAAAAT GCCAGATTAC
TTACTTTTCA4751 GACATGGCCA TTGACTTTTC TGTCGCCAAC AGATCTGGCA
CGAGCAGGCT4801 TTTACTACAT AGGACCTGGA GACAGAGTGG CTTGCTTTGC
CTGTGGTGGA4851 AAATTGAGCA ATTGGGAACC GAAGGATAAT GCTATGTCAG
AACACCTGAG4901 ACATTTTCCC AAATGCCCAT TTATAGAAAA TCAGCTTCAA
GACACTTCAA4951 GATACACAGT TTCTAATCTG AGCATGCAGA CACATGCAGC
CCGCTTTAAA5001 ACATTCTTTA ACTGGCCCTC TAGTGTTCTA GTTAATCCTG
AGCAGCTTGC5051 AAGTGCGGGT TTTTATTATG TGGGTAACAG TGATGATGTC
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TCCATGGGTT5151 CAACATGCCA AGTGGTTTCC AAGGTGTGAG TACTTGATAA
GAATTAAAGG5201 ACAGGAGTTC ATCCGTCAAG TTCAAGCCAG TTACCCTCAT
CTACTTGAAC5251 AGCTGCTATC CACATCAGAC AGCCCAGGAG ATGAAAATGC
AGAGTCATCA5301 ATTATCCATT TTGAACCTGG AGAAGACCAT TCAGAAGATG
CAATCATGAT

66/67

FIG. 16H

5351 GAATACTCCT GTGATTAATG CTGCCGTGGA AATGGGCTTT
AGTAGAAGCC

5401 TGGTAAAACA GACAGTTCAG AGAAAAATCC TAGCAACTGG
AGAGAATTAT

5451 AGACTAGTCA ATGATCTTGT GTTAGACTTA CTCAATGCAG
AAGATGAAAT

5501 AAGGGAAGAG GAGAGAGAAA GAGCAACTGA GGAAAAAGAA
TCAAATGATT

5551 TATTATTAAT CCGGAAGAAT AGAATGGCAC TTTTCAACA
TTTGACTTGT

5601 GTAATTCCAA TCCTGGATAG TCTACTAACT GCCGGAATTA
TTAATGAACA

5651 AGAACATGAT GTTATTAAAC AGAAGACACA GACGTCTTTA
CAAGCAAGAG

5701 AACTGATTGA TACGATTTTA GTAAAAGGAA ATATTGCAGC
CACTGTATTC

5751 AGAAACTCTC TGCAAGAAGC TGAAGCTGTG TTATATGAGC
ATTTATTTGT

5801 GCAACAGGAC ATAAAATATA TTCCACAGA AGATGTTTCA
GATCTACCAG

5851 TGGAAGAACA ATTGCGGAGA CTACAAGAAG AAAGAACATG
TAAAGTGTGT

5901 ATGGACAAAG AAGTGTCCAT AGTGTTTATT CCTTGTGGTC
ATCTAGTAGT

5951 ATGCAAAGAT TGTGCTCCTT CTTTAAGAAA GTGTCCTATT
TGTAGGAGTA

6001 CAATCAAGGG TACAGTTCGT ACATTTCTTT CATGAAGAAG
AACCAAAACA

6051 TCGTCTAAAC TTTAGAATTA ATTTATTAAA TGTATTATAA
CTTTAACTTT

67/67

FIG. 16I

6101 TATCCTAATT TGGTTTCCTT AAAATTTTTA TTTATTTACA
ACTCAAAAAA

6151 CATTGTTTTG TGTAACATAT TTATATATGT ATCTAAACCA
TATGAACATA

6201 TATTTTTTAG AAATAAGAG AATGATAGGC TTTGTTCCT
ATGAACGAAA

6251 AAGAGGTAGC ACTACAAACA CAATATTCAA TCAAAATTTC
AGCATTATTG

6301 AAATTGTAAG TGAAGTAAAA CTTAAGATAT TTGAGTTAAC
CTTTAAGAAT

6351 TTAAATATT TTGGCATTGT ACTAATACCG GGAACATGAA
GCCAGGTGTG

6401 GTGGTATGTG CCTGTAGTCC CAGGCTGAGG CAAGAGAATT
ACTTGAGCCC

6451 AGGAGTTTGA ATCCATCCTG GGCAGCATAC TGAGACCCTG
CCTTTAAAAA

6501 CAAACAGAAC AAAAACAAAA CACCAGGGAC ACATTTCTCT
GTCTTTTTTG

6551 ATCAGTGTCC TATACATCGA AGGTGTGCAT ATATGTTGAA
TCACATTTTA

6601 GGGACATGGT GTTTTTATAA AGAATTCTGT GAGAAAAAAT
TTAATAAAGC

6651 AACCAAAAAA AAAAAAAAAA

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gtcaaactcat taattagga 19

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19

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<400> 18

gttataggag ctaacaaat

19

<210> 19

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> based on Homo sapiens

<400> 19

aatgtgaaac acaagcaac

19

<210> 20

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<220>

<223> based on Homo sapiens

<400> 20

acattatatt aggaaatcc

19

<210> 21

<211> 19

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<223> based on Homo sapiens

<400> 21

cttgtccacc ttttctaaa

19

<210> 22

<211> 19

<212> DNA

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<220>

<223> based on Homo sapiens

<400> 22

atcttctctt gaaaatagg

19

<210> 23

<211> 19

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<400> 23

ccttcaaaac tgttaaaag

19

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<400> 24

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19

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atactttcct gtgtcttcc 19

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accattctgg ataccagaa 19

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agatggcctg tctaaggca 19

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ttcagttttc caccacaac 19

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tatcagaact cacagcatc

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<213> Artificial Sequence

<220>
<223> based on Homo sapiens

<400> 192
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<210> 193
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<212> DNA
<213> Artificial Sequence

<220>
<223> based on Homo sapiens

<400> 193
gggctcaagt aattctctt 19

<210> 194
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<220>
<223> based on Homo sapiens

<400> 194
gcccaggatg gattcaaac 19

<210> 195
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<222> 1
<223> y=gm

<221> modified_base
<222> 18
<223> y=cm

<400> 195
yagaagatga ctggtaya 19

<210> 196
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<221> misc_feature
<222> 1,17,18
<223> y=u or t

<400> 196
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<220>
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<400> 197
tctgcttcaa ggagctggaa 20

<210> 198
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<220>
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gaaaggaaag cgcaaccg 18

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gaagatggtg atgggattc 19

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caagcttccc gttctcagcc 20

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<223> based on Homo sapiens

<400> 209
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<223> y=cm

<221> modified_base
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<223> y=um

<223> based on Homo sapiens

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<210> 212

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<223> y=um

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<223> based on Homo sapiens

<400> 212
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<210> 213
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<400> 213
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<210> 214
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<220>
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<400> 214
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<210> 215
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cggagcgtga ggatggaga

19

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<221> VARIANT

<222> 1-3,6,9,10,14,15,18-20,24, 30,32,33,35,37,40, 42-47, 49-51,
53-57, 59-62, 64,66

<223> Xaa=any amino acid

<221> VARIANT

<222> 13, 16,17

<223> Xaa=any amino acid or is absent

<400> 216

Xaa	Xaa	Xaa	Arg	Leu	Xaa	Thr	Phe	Xaa	Xaa	Trp	Pro	Xaa	Xaa	Xaa	Xaa
1				5					10					15	
Xaa	Xaa	Xaa	Xaa	Xaa	Leu	Ala	Xaa	Ala	Gly	Phe	Tyr	Tyr	Xaa	Gly	Xaa
			20					25					30		
Xaa	Asp	Xaa	Val	Xaa	Cys	Phe	Xaa	Cys	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Trp
		35					40					45			
Xaa	Xaa	Xaa	Asp	Xaa	Xaa	Xaa	Xaa	Xaa	His	Xaa	Xaa	Xaa	Xaa	Pro	Xaa
	50					55					60				
Cys	Xaa	Phe	Val												
65															

<210> 217

<211> 46

<212> PRT

<213> Artificial Sequence

<220>

<223> based on Homo sapiens

<221> VARIANT

<222> 2-7,9-11,17-21,23,25, 30-32,34-35, 38-42 ,45

<223> Xaa=any amino acid

<221> VARIANT

<222> 8

<223> Xaa=Glu or Asp

<221> VARIANT

<222> 14,22

<223> Xaa=Val or Ile

<400> 217

Glu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Lys	Xaa	Cys	Met
1				5					10					15	
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Phe	Xaa	Pro	Cys	Gly	His	Xaa	Xaa	Xaa
			20					25					30		
Cys	Xaa	Xaa	Cys	Ala	Xaa	Xaa	Xaa	Xaa	Xaa	Cys	Pro	Xaa	Cys		
		35					40					45			

<210> 218

<211> 2540

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<222> (1)...(2540)

<223> n=a,t,c, or g

<400> 218

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ttaaaaactt ttgctaattt tccaagtggt agtcctgttt cagcatcaac actggcacga 180
gcagggtttc tttatactgg tgaaggagat accgtgcggt gctttagtgt tcatgcagct 240
gtagatagat ggcaatatgg agactcagca gttggaagac acaggaaagt atccccaagt 300
tgcagattta tcaacggcct tttcttgaa aatagtgcc cgcagctctac aaattctggt 360
atccagaatg gtcagtacaa agttgaaaac tatctgggaa gcagagatca ttttgcctta 420
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tactacacag gtattggtga ccaagtgcag tgctttgtt gtggtggaaa actgaaaaat 660
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<210> 219

<211> 497

<212> PRT

<213> Homo sapiens

<400> 219

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Ile Asn Lys Glu Glu Glu Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
          20             25             30

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Phe Ala Asn Phe Pro Ser Gly Ser Pro Val Ser Ala Ser Thr Leu Ala
 35 40 45
 Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Arg Cys Phe
 50 55 60
 Ser Cys His Ala Ala Val Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val
 65 70 75 80
 Gly Arg His Arg Lys Val Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
 85 90 95
 Tyr Leu Glu Asn Ser Ala Thr Gln Ser Thr Asn Ser Gly Ile Gln Asn
 100 105 110
 Gly Gln Tyr Lys Val Glu Asn Tyr Leu Gly Ser Arg Asp His Phe Ala
 115 120 125
 Leu Asp Arg Pro Ser Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
 130 135 140
 Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
 145 150 155 160
 Tyr Cys Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
 165 170 175
 Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
 180 185 190
 Gly Ile Gly Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
 195 200 205
 Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
 210 215 220
 Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Leu Asn Ile Arg Ser Glu
 225 230 235 240
 Ser Asp Ala Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Leu
 245 250 255
 Pro Arg Asn Pro Ser Met Ala Asp Tyr Glu Ala Arg Ile Phe Thr Phe
 260 265 270
 Gly Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly
 275 280 285
 Phe Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly
 290 295 300
 Gly Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Glu Gln His
 305 310 315 320
 Ala Lys Trp Tyr Pro Gly Cys Lys Tyr Leu Leu Glu Gln Lys Gly Gln
 325 330 335
 Glu Tyr Ile Asn Asn Ile His Leu Thr His Ser Leu Glu Glu Cys Leu
 340 345 350
 Val Arg Thr Thr Glu Lys Thr Pro Ser Leu Thr Arg Arg Ile Asp Asp
 355 360 365
 Thr Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe
 370 375 380
 Ser Phe Lys Asp Ile Lys Lys Ile Met Glu Glu Lys Ile Gln Ile Ser
 385 390 395 400
 Gly Ser Asn Tyr Lys Ser Leu Glu Val Leu Val Ala Asp Leu Val Asn
 405 410 415
 Ala Gln Lys Asp Ser Met Gln Asp Glu Ser Ser Gln Thr Ser Leu Gln
 420 425 430
 Lys Glu Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys
 435 440 445
 Leu Cys Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Val Pro
 450 455 460
 Cys Gly His Leu Val Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys
 465 470 475 480
 Cys Pro Met Cys Tyr Thr Val Ile Thr Phe Lys Gln Lys Ile Phe Met
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Ser

<210> 220
 <211> 2676
 <212> DNA
 <213> Homo sapiens

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 <223> n=a,t,c, or g

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 gcaaagccat gcacaaaact acctccctag agaaaggcta gtcccttttc ttccccattc 180
 attttcattat gaacatagta gaaaacagca tattcttatc aaatttgatg aaaagcgcca 240
 acacgtttga actgaaatac gacttgtcat gtgaactgta ccgaatgtct acgtattcca 300
 cttttcctgc tggggttcct gtctcagaaa ggagtcttgc tctgtctggt ttctattaca 360
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 gaggagacag tcctactgaa aagcataaaa agttgtatcc tagctgcaga ttctgttcaga 480
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 <213> Homo sapiens

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 35 40 45
 Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys Val
 50 55 60
 Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Arg Gly Asp
 65 70 75 80
 Ser Pro Thr Glu Lys His Lys Lys Leu Tyr Pro Ser Cys Arg Phe Val
 85 90 95
 Gln Ser Leu Asn Ser Val Asn Asn Leu Glu Ala Thr Ser Gln Pro Thr
 100 105 110
 Phe Pro Ser Ser Val Thr His Ser Thr His Ser Leu Leu Pro Gly Thr
 115 120 125
 Glu Asn Ser Gly Tyr Phe Arg Gly Ser Tyr Ser Asn Ser Pro Ser Asn
 130 135 140
 Pro Val Asn Ser Arg Ala Asn Gln Glu Phe Ser Ala Leu Met Arg Ser
 145 150 155 160
 Ser Tyr Pro Cys Pro Met Asn Asn Glu Asn Ala Arg Leu Leu Thr Phe
 165 170 175
 Gln Thr Trp Pro Leu Thr Phe Leu Ser Pro Thr Asp Leu Ala Arg Ala
 180 185 190
 Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys
 195 200 205
 Gly Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asn Ala Met Ser Glu
 210 215 220
 His Leu Arg His Phe Pro Lys Cys Pro Phe Ile Glu Asn Gln Leu Gln
 225 230 235 240
 Asp Thr Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala
 245 250 255
 Ala Arg Phe Lys Thr Phe Phe Asn Trp Pro Ser Ser Val Leu Val Asn
 260 265 270
 Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Asn Ser Asp
 275 280 285
 Asp Val Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser
 290 295 300
 Gly Asp Asp Pro Trp Val Gln His Ala Lys Trp Phe Pro Arg Cys Glu
 305 310 315 320
 Tyr Leu Ile Arg Ile Lys Gly Gln Glu Phe Ile Arg Gln Val Gln Ala
 325 330 335
 Ser Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro
 340 345 350
 Gly Asp Glu Asn Ala Glu Ser Ser Ile Ile His Leu Glu Pro Gly Glu
 355 360 365
 Asp His Ser Glu Asp Ala Ile Met Met Asn Thr Pro Val Ile Asn Ala
 370 375 380
 Ala Val Glu Met Gly Phe Ser Arg Ser Leu Val Lys Gln Thr Val Gln

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385          390          395          400
Arg Lys Ile Leu Ala Thr Gly Glu Asn Tyr Arg Leu Val Asn Asp Leu
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Val Leu Asp Leu Leu Asn Ala Glu Asp Glu Ile Arg Glu Glu Glu Arg
          420          425          430
Glu Arg Ala Thr Glu Glu Lys Glu Ser Asn Asp Leu Leu Ile Arg
          435          440          445
Lys Asn Arg Met Ala Leu Phe Gln His Leu Thr Cys Val Ile Pro Ile
          450          455          460
Leu Asp Ser Leu Leu Thr Ala Gly Ile Ile Asn Glu Gln Glu His Asp
465          470          475          480
Val Ile Lys Gln Lys Thr Gln Thr Ser Leu Gln Ala Arg Glu Leu Ile
          485          490          495
Asp Thr Ile Leu Val Lys Gly Asn Ile Ala Ala Thr Val Phe Arg Asn
          500          505          510
Ser Leu Gln Glu Ala Glu Ala Val Leu Tyr Glu His Leu Phe Val Gln
          515          520          525
Gln Asp Ile Lys Tyr Ile Pro Thr Glu Asp Val Ser Asp Leu Pro Val
          530          535          540
Glu Glu Gln Leu Arg Arg Leu Pro Glu Glu Arg Thr Cys Lys Val Cys
545          550          555          560
Met Asp Lys Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val
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Val Cys Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg
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cacaaaactg cctoccaaag acttttccca ggtccctcgt atcaaaacat taagagtata 300
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<210> 223

<211> 618

<212> PRT

<213> Homo sapiens

<400> 223

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Asn Ser Asn Lys Gln Lys Met Lys Tyr Asp Phe Ser Cys Glu Leu Tyr
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Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro Val Ser Glu
50     55     60
Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys
65     70     75     80
Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Leu Gly
85     90     95
Asp Ser Pro Ile Gln Lys His Lys Gln Leu Tyr Pro Ser Cys Ser Phe
100    105    110
Ile Gln Asn Leu Val Ser Ala Ser Leu Gly Ser Thr Ser Lys Asn Thr
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Ser Pro Met Arg Asn Ser Phe Ala His Ser Leu Ser Pro Thr Leu Glu
130    135    140
His Ser Ser Leu Phe Ser Gly Ser Tyr Ser Ser Leu Pro Pro Asn Pro
145    150    155    160
Leu Asn Ser Arg Ala Val Glu Asp Ile Ser Ser Ser Arg Thr Asn Pro
165    170    175
Tyr Ser Tyr Ala Met Ser Thr Glu Glu Ala Arg Phe Leu Thr Tyr His
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Met Trp Pro Leu Thr Phe Leu Ser Pro Ser Glu Leu Ala Arg Ala Gly

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Arg	Arg	His	Phe	Pro	Asn	Cys	Pro	Phe	Leu	Glu	Asn	Ser	Leu	Glu	Thr
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Asp	Pro	Trp	Val	Glu	His	Ala	Lys	Trp	Phe	Pro	Arg	Cys	Glu	Phe	Leu
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Pro	His	Leu	Leu	Glu	Gln	Leu	Leu	Ser	Thr	Ser	Asp	Thr	Thr	Gly	Glu
		355					360					365			
Glu	Asn	Ala	Asp	Pro	Pro	Ile	Ile	His	Phe	Gly	Pro	Gly	Glu	Ser	Ser
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Ser	Glu	Asp	Ala	Val	Met	Met	Asn	Thr	Pro	Val	Val	Lys	Ser	Ala	Leu
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Glu	Met	Gly	Phe	Asn	Arg	Asp	Leu	Val	Lys	Gln	Thr	Val	Leu	Ser	Lys
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Ile	Leu	Thr	Thr	Gly	Glu	Asn	Tyr	Lys	Thr	Val	Asn	Asp	Ile	Val	Ser
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Ala	Leu	Leu	Asn	Ala	Glu	Asp	Glu	Lys	Arg	Glu	Glu	Glu	Lys	Glu	Lys
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Gln	Ala	Glu	Glu	Met	Ala	Ser	Asp	Asp	Leu	Ser	Leu	Ile	Arg	Lys	Asn
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Arg	Met	Ala	Leu	Phe	Gln	Gln	Leu	Thr	Cys	Val	Leu	Pro	Ile	Leu	Asp
465					470					475					480
Asn	Leu	Leu	Lys	Ala	Asn	Val	Ile	Asn	Lys	Gln	Glu	His	Asp	Ile	Ile
				485					490					495	
Lys	Gln	Lys	Thr	Gln	Ile	Pro	Leu	Gln	Ala	Arg	Glu	Leu	Ile	Asp	Thr
			500					505					510		
Ile	Trp	Val	Lys	Gly	Asn	Ala	Ala	Ala	Asn	Ile	Phe	Lys	Asn	Cys	Leu
		515				520						525			
Lys	Glu	Ile	Asp	Ser	Thr	Leu	Tyr	Lys	Asn	Leu	Phe	Val	Asp	Lys	Asn
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<210> 224
<211> 2100
<212> DNA

<213> Mus musculus

<400> 224

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<210> 225

<211> 496

<212> PRT

<213> Mus musculus

<400> 225

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 20             25             30
Phe Ala Asn Phe Pro Ser Ser Ser Pro Val Ser Ala Ser Thr Leu Ala
 35             40             45
Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Gln Cys Phe
 50             55             60
Ser Cys His Ala Ala Ile Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val
 65             70             75             80
Gly Arg His Arg Arg Ile Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
 85             90             95

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Gly Gln Tyr Lys Ser Glu Asn Cys Val Gly Asn Arg Asn Pro Phe Ala
      115      120      125
Pro Asp Arg Pro Pro Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
      130      135      140
Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
      145      150      155      160
Cys Ser Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
      165      170      175
Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
      180      185      190
Gly Ala Asp Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
      195      200      205
Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
      210      215      220
Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Val Asn Val Arg Ser Glu
      225      230      235      240
Ser Gly Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Ser Pro
      245      250      255
Arg Asn Pro Ala Met Ala Glu Tyr Glu Ala Arg Ile Val Thr Phe Gly
      260      265      270
Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly Phe
      275      280      285
Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly Gly
      290      295      300
Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Asp Gln His Ala
      305      310      315      320
Lys Cys Tyr Pro Gly Cys Lys Tyr Leu Leu Asp Glu Lys Gly Gln Glu
      325      330      335
Tyr Ile Asn Asn Ile His Leu Thr His Pro Leu Glu Glu Ser Leu Gly
      340      345      350
Arg Thr Ala Glu Lys Thr Pro Pro Leu Thr Lys Lys Ile Asp Asp Thr
      355      360      365
Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe Ser
      370      375      380
Phe Lys Asp Leu Lys Lys Thr Met Glu Glu Lys Ile Gln Thr Ser Gly
      385      390      395      400
Ser Ser Tyr Leu Ser Leu Glu Val Leu Ile Ala Asp Leu Val Ser Ala
      405      410      415
Gln Lys Asp Asn Thr Glu Asp Glu Ser Ser Gln Thr Ser Leu Gln Lys
      420      425      430
Asp Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Lys Leu
      435      440      445
Ser Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Phe Pro Cys
      450      455      460
Gly His Leu Ala Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys
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<210> 226

<211> 2474

<212> DNA

<213> Mus musculus

<400> 226

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<210> 227

<211> 602

<212> PRT

<213> Mus musculus

<400> 227

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Leu Ser Thr Tyr Ser Ala Phe Pro Arg Gly Val Pro Val Ser Glu Arg
          35             40             45
Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Ala Asn Asp Lys Val
          50             55             60
Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Gln Gly Asp

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			100					105					110	
Leu	Pro	Ser	Thr	Ala	Met	Ser	Thr	Met	Pro	Leu	Ser	Phe	Ala	Ser
		115					120					125		
Glu	Asn	Thr	Gly	Tyr	Phe	Ser	Gly	Ser	Tyr	Ser	Ser	Phe	Pro	Ser
	130					135				140				Asp
Pro	Val	Asn	Phe	Arg	Ala	Asn	Gln	Asp	Cys	Pro	Ala	Leu	Ser	Thr
145					150				155					160
Pro	Tyr	His	Phe	Ala	Met	Asn	Thr	Glu	Lys	Ala	Arg	Leu	Leu	Thr
			165					170					175	Tyr
Glu	Thr	Trp	Pro	Leu	Ser	Phe	Leu	Ser	Pro	Ala	Lys	Leu	Ala	Lys
		180						185					190	Ala
Gly	Phe	Tyr	Tyr	Ile	Gly	Pro	Gly	Asp	Arg	Val	Ala	Cys	Phe	Ala
	195					200						205		Cys
Asp	Gly	Lys	Leu	Ser	Asn	Trp	Glu	Arg	Lys	Asp	Asp	Ala	Met	Ser
	210					215				220				Glu
His	Gln	Arg	His	Phe	Pro	Ser	Cys	Pro	Phe	Leu	Lys	Asp	Leu	Gly
225					230				235					240
Ser	Ala	Ser	Arg	Tyr	Thr	Val	Ser	Asn	Leu	Ser	Met	Gln	Thr	His
			245					250					255	Ala
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		340						345				350		
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Gln	Ile	Leu	Ala	Thr	Gly	Glu	Asn	Tyr	Arg	Thr	Val	Ser	Asp	Leu
			405						410					415
Ile	Gly	Leu	Leu	Asp	Ala	Glu	Asp	Glu	Met	Arg	Glu	Glu	Gln	Met
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Ile	Arg	Ser	Leu	Pro	Thr	Asp	Asp	Ile	Ala	Ala	Leu	Pro	Met	Glu
														Glu

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 450 455 460
 Glu His Asp Ile Ile Arg Gln Lys Thr Gln Ile Pro Leu Gln Ala Arg
 465 470 475 480
 Glu Leu Ile Asp Thr Val Leu Val Lys Gly Asn Ala Ala Ala Asn Ile
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